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(54) Title: DIAGNOSTIC OR THERAPEUTIC SOMATOSTATIN OR BOMBESIN ANALOG CONJUGATES AND USES THEREOF

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(57) Abstract: A probe apparatus for self-administered treatment of prostatitis has a pair of opposing handle bars (12) symmetrical to each other having handle grip ends (10, 11) and connected to a center housing (4) along a common axis, and a probe (1) connected to a front side of the center housing and extending to a probe tip (3). The center housing has a vibrator motor which is mechanically coupled to the probe tip, and is actuated by a switch on one of the handle grip ends. The probe has a swivelable tip actuated by a pair of swivel actuators (6, 7) provided on the handle grip ends. The probe tip has an off-center bulbous or ovoid shape for effecting a sweeping movement over the prostate when a swivel actuator is actuated. The probe and the handle grip ends have approximately the same length and extend in the same direction for more precise manipulation by the patient. The handle bars are connected to the center housing by twist-lock disconnectors (14) to be detachable for storage or packing. The probe apparatus is employed by inserting the probe into the rectum from the rear of the patient, and positioning the probe tip adjacent the colon walls proximate the prostate while manipulating the handle grip ends on each side of the patient. It is employed with the patient in a standing, bent over position, and a mirror is placed on the floor between the patient's feet to provide visual confirmation of the position of the probe. The probe apparatus is effective for prostatic drainage by manipulating the swivel actuators to sweep over the surface areas of the prostate toward the prostate center in order to drain fluid from the prostate gland and ducts.

DIAGNOSTIC OR THERAPEUTIC SOMATOSTATIN OR BOMBESIN
5 ANALOG CONJUGATES AND USES THEREOF

Field of the Invention

The invention relates to somatostatin and bombesin analogs and uses thereof for targeting compounds useful in the detection, diagnosis or treatment of disease.

Background

The toxic side effects of many therapies, including standard treatments for cancers, effectively limit the amount of agent that may be administered to a patient. Additionally, many agents cause organ-specific toxicities, further limiting the dose that may be delivered to the target tissue. For instance, the cardiotoxicity of many anthracycline family members reduces the maximum therapeutic dose available for this group of chemotherapeutic agents. Targeted drug delivery of various detectable or therapeutic agents can lower toxicity in normal tissue and increase the efficacy of treatment by allowing concentrated localized effects on specific tissues.

Somatostatin, bombesin, or other biologically active peptide analogs have been used to detect tumor cells over expressing receptors specific for the peptides (see for example Denzler and Reubi, *Cancer* 85(1):188-198, 1999).
25 Somatostatin, bombesin, and many other biologically active peptide agonist analogs are rapidly internalized after binding to their receptors (Lukinius et al., *Acta Onc.* 38:383-387, 1999; Morel, *Biochem. Pharmacol.* 47(1):63-76, 1994). This internalization of the peptide analogs may result in translocation to the cell nucleus (Chen et al., *Am. J. Physiol. Renal Physiol.* 279:F440-F448, 2000; 30 Hornick et al., *J. Nucl. Med.* 41(7):1256-1263, 2000; Janson et al., *J. Nucl. Med.* 41(9):1514-1518, 2000).

Somatostatin analogs bind particular somatostatin receptor subtypes that are present on the surface of specific normal or diseased tissues. Somatostatin receptors are up-regulated in specific diseased tissues including inflammatory bowel diseases, rheumatoid arthritis, a variety of tumor types, and blood vessels supplying many tumors, often in a subtype-specific manner. (Denzler and Reubi, *Cancer*, 85:188-198, 1999; Plonowski et al., *Cancer Res.* 60(11):2996-3001, 2000; Kahan et al., *Int. J. Cancer* 82(4):592-598, 1999; Gulec et al., *Surg. Res.* 97(2):131-137, 2001). Similarly, receptors specific for another biologically active peptide, substance P, can be up-regulated in various diseases (Id.). Somatostatin-related urotensin II peptide receptors have been found to be expressed on a number of neural tumors (Takahashi et al., *Peptides* 22:1175-1179, 2001). Receptors for GnRH II ligands/analogs have been located on many peripheral tissues of interest including breast, prostate, and the GI tract (Neill et al., *Biochem. Biophys. Res. Commun.* 282:1012-1019; Millar et al., *Proc. Natl. Acad. Sci. USA* 98:963609641, 2001).

At least five somatostatin receptors subtypes have been characterized, and tumors can express various receptor subtypes (Shaer et al., *Int. J. Cancer* 70:530-537, 1997). Naturally occurring somatostatin and its analogs exhibit differential binding to these receptor subtypes, allowing precise targeting of a peptide analog to specific diseased tissues.

The physical and chemical properties of many compounds such as cytotoxic agents make their conjugation to biologically active peptides problematic. The agent or drug may reduce the specificity of binding or the biological activity of the peptide analog, limiting its effectiveness as a targeting agent. Additionally, therapeutic and cytotoxic agents may have chemical properties that cause reduced solubility and promote accumulation of drug-peptide analogs in certain organs, thus increasing toxicity and reducing efficacy. Effective means are needed to link cytotoxic agents to a targeting agent such as somatostatin, bombesin, or another biologically active peptide

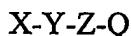
and to decrease non-target uptake of the cytotoxic agents, while retaining the activity of each component, thus maximizing therapeutic effects and minimizing toxicity.

Furthermore, while iodination and astatination (see Vaidyanathan et 5 al., *Nucl. Med. Biol.* 27(4):329-337, 2000) hold great promise for use in imaging and possibly in aiding treatment of diseases associated with increased expression of a factor specific for a biologically active peptide, problems exist with the methods now available for labeling a range of peptide analogs. The use of labeled biologically active peptides has been studied in various systems. 10 Radioactive halogens such as iodine have great potential as tumor imaging and cytotoxic agents. Promising isotopes include ¹²⁵I (K.S. Sastry, *Am. Assoc. Phys. Med.* 19:1361-1370, 1992; Mariano, *J. Nucl. Med.* 41(9):1519-1521, 2000), ¹³¹I (Wheldon et al., *Radiother. Oncol.*, 21:91-99, 1991), ¹²³I (Blower et al., *Eur. J. Nucl. Med.* 25:101-108, 1998; Janson et al, *J. Nuc. Med.* 15 41(9):1514-1518, 2000; Mariani et al, *J. Nuc. Med.* 41(9):1519-1521, 2000), and ¹²⁴I (Glaser et al., *J. Labelled Compd. Radiopharm.* 44(6):465-480, 2001). There are several problems associated with the addition of radioactive iodine atoms to peptides (Bakker et al., *Eur. J. Nucl. Med.* 23(7):775-781, 1996). One is the rapid loss of iodines from L-Tyr residues by specific de-iodination 20 enzymes (Kawai et al., *Nucl. Med. Biol.* 17(4):369-76, 1990). Another problem is the great increase in hydrophobicity produced by addition of iodine to a peptide agent, which is associated with increased accumulation of radioactivity in the liver, interfering with tumor imaging and promoting severe toxicity. A further problem is loss of binding affinity when tyrosines next to the 25 pharmacophore are iodinated. A linker capable of facilitating labeling of a variety of biologically active peptides without deleterious *in vivo* accumulation is needed.

Summary of Invention

The present invention features biologically active peptides such as somatostatin or bombesin conjugated to chemical compounds through linkers that allow retention of the peptide's biological activity. Such peptide agents 5 are useful for specifically targeting therapeutic agents, cytotoxic agents, or detectable labels to cells, such as cancer cells expressing somatostatin or bombesin receptors.

In a first aspect, the invention features a peptide agent of the formula:



10 wherein X is optionally selected from cytotoxic agents, therapeutic agents, detectable labels or chelating groups; Y is a peptide that increases the biodistribution of the peptide agent, a hydrophilic polymer that includes a linker for X, or is omitted; Q is a biologically active peptide such as somatostatin or bombesin, and Z is a linking peptide that, when bonded to Q at 15 the N-terminus or at a compatible side-chain amino group of Q, preserves at least 50% of the biological activity of Q. Z has the formula: A-B-C-E-F, where A is D-Lys, D-Tyr, D-Ser, or L-Ser, or deleted; B is D-Lys or D-Tyr, or is deleted; C is Lys, Ser, hSer, Thr, Nle, Abu, Nva, (2,3, or 4) 3-pyridyl-Ala (Pal), Orn, Dab, Dap, 4-NH₂-Phe, D-4-OH-Pro, or L-4-OH-Pro, or is deleted; E 20 is D-Lys, D-Tyr, D-Ser, D-4-OH-Pro, L-4-OH-Pro, 3-iodo-D-Tyr, 3-5 diido-D-Tyr, 3-astatine-D-Tyr, 3-5 astatine-D-Tyr, 3-bromo-D-Tyr, 3-5 dibromo-D-Tyr, D-Asn, L-Asn, D-Asp, L-Asp, D-Glu, L-Glu, D-Gln, or L-Gln; and F is D-Lys, D-Tyr, D-Ser, L-Ser, D-4-OH-Pro, L-4-OH-Pro, 3-iodo-D-Tyr, 3-5 diido-D-Tyr, 3-astatine-D-Tyr, 3-5 astatine-D-Tyr, 3-bromo-D-Tyr, 3-5 25 dibromo-D-Tyr, D-Asn, L-Asn, D-Asp, L-Asp, D-Glu, L-Glu, D-Gln, or L-Gln; provided that when A, B, C, and E are Tyr, Tyr, Lys, and Tyr, respectively, F is not Lys; and when A, B, C, and E, are Lys, Tyr, Lys, and Tyr, respectively, E is not Tyr or Lys; and when A and B are deleted, and C and E are Lys and Tyr, respectively, F is not Tyr or Lys.

In a second, related aspect, the invention features a peptide agent having the formula:

X-Y-Z-Q

wherein: X is a cytotoxic or therapeutic agent; Y is a peptide that increases the 5 hydrophilic distribution of the peptide agent, a hydrophilic polymer that includes a linker for X, or is omitted; Q is a peptide having biological activity; and Z is a linking peptide that when bonded to Q at the N-terminus or at a compatible side-chain amino group of Q, preserves at least 50% of the biological activity of Q, wherein Z has the formula:

10 A-B-C-E-F

where A is D-Lys, D-Tyr, D-Ser, or L-Ser, or is deleted; B is D-Lys or D-Tyr, or is deleted; C is Lys, Ser, hSer, Thr, Nle, Abu, Nva, (2, 3, or 4) 3-pyridyl-Ala (Pal), Orn, Dab, Dap, 4-NH₂-Phe, D-4-OH-Pro, or L-4-OH-Pro, or is deleted; E is D-Lys, D-Tyr, D-Ser, D-4-OH-Pro, L-4-OH-Pro, 3-iodo-D-Tyr, 3-5 diiodo-D-Tyr, 3-astatine-D-Tyr, 3-5 astatine-D-Tyr, 3-bromo-D-Tyr, 3-5 dibromo-D-Tyr, D-Asn, L-Asn, D-Asp, L-Asp, D-Glu, L-Glu, D-Gln, or L-Gln; and F is D-Lys, D-Tyr, D-Ser, L-Ser, D-4-OH-Pro, L-4-OH-Pro, 3-iodo-D-Tyr, 3-5 diiodo-D-Tyr, 3-astatine-D-Tyr, 3-5 astatine-D-Tyr, 3-bromo-D-Tyr, 3-5 dibromo-D-Tyr, D-Asn, L-Asn, D-Asp, L-Asp, D-Glu, L-Glu, D-Gln, or L-Gln; provided that when A, B, C, and E are Tyr, Tyr, Lys, and Tyr, respectively, F is not Lys; and when A, B, C, and E are Lys, Tyr, Lys, and Tyr, respectively, E is not Tyr or Lys; and when A and B are deleted, and C and E are Lys and Tyr, respectively, F is not Tyr or Lys. The Z peptide linker may thus be 2, 3, 4, or 5 residues in length.

25 In one embodiment of the first and second aspects of the invention, X is a cytotoxic agent or cytotoxic agents. Preferably, X is an antimetabolic agent. The cytotoxic agent may be selected from the group consisting of: doxorubicin, methotrexate, camptothecin, homocamptothecins, rhizoxins, dolistatins, paclitaxel, combretastatin, and maytansinoids, or derivatives or analogs thereof.

30 For example, the cytotoxic agent methotrexate may be linked through the linker

Z or Y-Z to a peptide analog, forming a peptide agent of the invention.

Preferred cytotoxic agents are rhizoxin, rhizoxin-D, camptothecin and its active analogs, homocamptothecin, ansamitocin P-3, dolistatins, epothilones, combretastatins, and combretastatin A-4.

5 In other preferred embodiments, the linking peptide Z is D-Ser-Nle-D-Ser-D-Ser, D-Ser-Lys-D-Ser-D-Ser, D-Ser-Lys-D-Tyr-D-Tyr, D-Ser-Lys-D-Tyr-D-Ser, D-Ser-Ser-D-Lys-D-Ser, D-Ser-Ser-D-Lys-Ser, D-Ser-Nle-D-Tyr-D-Ser, D-Ser-Pal-D-Tyr-D-Ser, D-Ser-Thr-D-Tyr-D-Ser, Lys-D-Ser-D-Ser, Ser-D-Lys-D-Ser, Ser-D-Lys-Ser, Nle-D-Tyr-D-Ser, Lys-D-Tyr-D-Ser, Pal-D-10 Lys-D-Ser, Thr-D-Tyr-D-Ser, D-Ser-D-Lys, D-Ser-D-Tyr, D-Lys-D-Lys, D-Lys-D-Tyr, or D-Tyr-D-Lys.

In a third aspect of the invention, the peptide agent has the formula:

X-Y-Z-Q,

where X is a chelating group, or is omitted; Y is a peptide that increases the 15 hydrophilic biodistribution of the peptide agent, a hydrophilic polymer that includes a linker for X, or is omitted; Q is a peptide having biological activity; Z is a linking peptide that, when bonded to Q at the N-terminus or at a compatible side-chain amino group of Q, preserves at least 50% of the biological activity of Q. In this aspect of the invention, Z has the formula: C-20 E-F, where C is Lys, Orn, Dab, Dap, 4- NH₂-Phe, Nle, Ser, hSer, Abu, Nva, D-4-OH-Pro, or L-4-OH-Pro, or is deleted; and E and F are each independently selected from the group consisting of: D-Lys, D-Tyr, D-Ser, D-4-OH-Pro, L-4-OH-Pro, 3-iodo-D-Tyr, 3-5 diiodo-D-Tyr, 3-bromo-D-Tyr, 3-5 dibromo-D-Tyr, D-Asn, D-Asp, L-Asp, D-Glu, or L-Glu; and when C and E are Lys and D-Tyr, 25 respectively, F is not D-Tyr or D-Lys.

In alternative embodiments of the third aspect, the peptide agents of the invention are attached to a detectable label directly or indirectly. The detectable label is preferably radioactive, such as radiolabeled iodine, astatine, or bromine bonded to an amino acid residue of the agent. Alternatively, X may

be a chelating group and may contain, for example, an isotope of Lu, In, Y, or Sm, or X may be omitted. If X is omitted, Y may be lower acetylated, succinylated, maleinylated or fumarylated.

In multiple embodiments of the third aspect, the invention features a
5 peptide agent in which Z is:

Lys-D-Tyr-D-Ser, Lys-D-Ser-D-Ser, or Nle-D-Tyr-D-Ser.

In another embodiment of the third aspect of the invention, Z may be three amino acids in length. Z is preferably the peptide agent of the formula:

C-E-F,

10 where C is selected from the amino acids listed as above, E is D-Tyr and F is D-Ser. Alternatively, Z may be Lys-D-Tyr-D-Ser, Lys-D-Ser-D-Ser, or Nle-D-Tyr-D-Ser.

In another embodiment of the third aspect, the invention features compositions in which Z has the formula:

15 C-E-F

where C is selected from the group consisting of Lys, Orn, Dab, Dap, 4-NH₂-Phe, Nle, Ser, hSer, Abu, Nva, D-4-OH-Pro, and L-4-OH-Pro, or is deleted; E is D-Tyr; and F is D-Ser.

In the first, second, and third aspects of the invention, Y may be a
20 peptide sequence that increases the hydrophilic biodistribution of the biologically active peptide conjugate. For example, in a preferred embodiment, Y is of the formula U(V-V)_n, wherein U is D-Pro, L-Pro, D-4-OH-Pro, L-4-OH-Pro, Sarcosine, Lys, Orn, Dab, Dap, 4-NH₂-Phe, or (NH₂-(CH₂)_m-COOH), where m=2-10, inclusive, or is deleted; each V is independently selected from
25 the group consisting of: D-Ser, L-Ser, D-Thr, L-Thr, D-Gln, L-Gln, D-Asn, L-Asn, D-4-OH-Pro, or L-4 hydroxy-Pro; and n=1-50, inclusive. In another preferred embodiment, each V is independently D-Ser or L-Ser. In another preferred embodiment, at least one V is a D-amino acid.

In the alternative, Y may be a hydrophilic polymer. For example, Y
30 may be polyethylene glycol, polyvinyl acetate, polyvinyl alcohol, HPMA (N-

(2-hydroxypropyl) methacrylamide) or HPMA copolymers, α , β -poly(N-hydroxyethyl)-DL-aspartamide (PHEA), α , β -poly(N-hydroxypropyl)-DL-aspartamide, or polyvinyl acetate.

The biologically active peptide Q is preferably a somatostatin peptide or
5 a bombesin peptide.

In one embodiment of the invention, Q is bombesin and Z has the formula:

E-F

where E is D-Lys, D-Tyr, D-Ser, D-4-OH-Pro, L-4-OH-Pro, 3-iodo-D-Tyr, 3-5
10 diiodo-D-Tyr, 3-astatine-D-Tyr, 3-5 astatine-D-Tyr, 3-bromo-D-Tyr, 3-5
dibromo-D-Tyr, D-Asn, L-Asn, D-Asp, L-Asp, D-Glu, L-Glu, D-Gln, or L-
Gln; and F is D-Lys, D-Tyr, D-Ser, L-Ser, D-4-OH-Pro, L-4-OH-Pro, 3-iodo-
D-Tyr, 3-5 diiodo-D-Tyr, 3-astatine-D-Tyr, 3-5 astatine-D-Tyr, 3-bromo-D-
Tyr, 3-5 dibromo-D-Tyr, D-Asn, L-Asn, D-Asp, L-Asp, D-Glu, L-Glu, D-Gln,
15 or L-Gln.

The present invention also provides methods of treating or diagnosing a disease comprising administering to a subject suffering from said disease a therapeutically effective amount of the peptide agents of the invention. For example tumors, or their angiogenic vessels, of the lung, breast, brain, eye, 20 prostate, or colon, the corresponding angiogenic blood vessels, or tumors of neuroendocrine origin, for example carcinoid syndrome, may be treated with a peptide agent.

Other features and advantages of the invention will be apparent from the following detailed description and from the claims.

25

Definitions

In accordance with the present invention, by "therapeutic agent" is meant any compound that is used in the detection, diagnosis or treatment of human disease. Such compounds may be naturally occurring, modified, or
30 synthetic. Therapeutic agents may promote or inhibit any biological process

implicated in a human disease pathway. Preferred disease targets include, but are not limited to, inflammatory bowel disease, rheumatoid arthritis, neoplastic cells or aberrantly proliferating cells, carcinoid syndrome, acromegaly, and angiogenesis. A therapeutic agent may be, for example, antineoplastic, 5 including cytotoxic. Antineoplastic agents may be alkylating agents, antibiotics, antimetabolites, hormonal agonists or antagonists, tubulin inhibitors, topoisomerase I and II inhibitors, or immunomodulators. They may operate through other mechanistic pathways, or antineoplastic agents may be supplementary potentiating agents.

10 By "cytotoxic agent" is meant any naturally occurring, modified, or synthetic compound that is toxic to tumor cells. Such agents are useful in the treatment of neoplasms, as well as inflammatory diseases, autoimmune disorders, and in the treatment of other symptoms or diseases characterized by cell proliferation or a hyperactive cell population. Cytotoxic agents include, 15 but are not limited to, alkylating agents, antibiotics, antimetabolites, tubulin inhibitors, topoisomerase I and II inhibitors, hormonal agonists or antagonists, or immunomodulators. They may also be cytotoxic when activated by light or infrared (Photofrin, IR dyes; *Nat. Biotechnol.* 19(4):327-331, 2001). They may operate through other mechanistic pathways, or cytotoxic agents may also be 20 supplementary potentiating agents.

By "detectable label" is meant any type of label which, when attached to a peptide agent, renders the compound detectable. A detectable label may be toxic or non-toxic, and may have one or more of the following attributes, without restriction: fluorescence (Kiefer et al., WO 9740055), color, toxicity 25 (e.g., radioactivity, e.g., a γ -emitting radionuclide, Auger-emitting radionuclide, β -emitting radionuclide, an α -emitting radionuclide, or a positron-emitting radionuclide), radiosensitivity, or photosensitivity. Although a detectable label may be directly attached to an amino acid residue of an analog of the invention, a detectable label may also be indirectly attached, for example, by being 30 complexed with a chelating group that is attached (e.g., linked via a covalent

bond or indirectly linked) to an amino acid residue of an analog. A detectable label may also be indirectly attached to an analog by the ability of the label to be specifically bound by a second molecule. One example of this type of an indirectly attached label is a biotin label that can be specifically bound by the 5 second molecule, streptavidin. The second molecule may also be linked to a moiety that allows neutron capture (e.g., a boron cage as described in, for example, Kahl et al., *Proc. Natl. Acad. Sci. USA* 87:7265-7269, 1990).

A detectable label may also be a metal ion from heavy elements or rare earth ions, such as Gd^{3+} , Fe^{3+} , Mn^{3+} , or Cr^{2+} (see, for example, *Invest. Radiol.* 10 33(10):752-761, 1998). Preferred radioactive detectable labels are radioactive iodine labels (e.g., ^{122}I , ^{123}I , ^{124}I , ^{125}I , or ^{131}I) that are capable of being coupled to each D- or L-Tyr or D- or L-4-amino-Phe residue present in the analogs of the invention. Preferred non-radioactive detectable labels are the many known dyes that are capable of being coupled to NH_2 -terminal amino acid residues.

Preferred examples of detectable labels that may be toxic to cells include ricin, diphtheria toxin, and radioactive detectable labels (e.g., ^{122}I , ^{123}I , ^{124}I , ^{125}I , ^{131}I , ^{177}Lu , ^{64}Cu , ^{67}Cu , ^{153}Sm , ^{166}Ho , ^{186}Re , ^{188}Re , ^{211}At , ^{212}Bi , ^{225}Ac , ^{67}Ga , ^{68}Ga , ^{75}Br , ^{76}Br , ^{77}Br , ^{117m}Sn , ^{47}Sc , ^{109}Pd , ^{89}Sr , ^{159}Gd , ^{149}Pm , ^{142}Pr , ^{111}Ag , ^{165}Dy , ^{213}Bi , ^{111}In , ^{114m}In , ^{201}Ti , ^{195m}Pt , ^{193}Pt , ^{86}Y and ^{90}Y). These compounds, 20 and others described herein may be directly or indirectly attached to a biologically active peptide or its analogs. A toxic detectable label may also be a chemotherapeutic agent (e.g., camptothecins, homocamptothecins, 5-fluorouracil or adriamycin), or may be a radiosensitizing agent (e.g., Taxol, gemcitabine, fluoropyrimidine, metronitozil, or the deoxycytidine analog 2',2' 25 difluoro- 2'-deoxycytidine (dFdCyd) to which is directly or indirectly attached a somatostatin analog of the present invention.

By "chelating group" is meant any group covalently bound to the peptide agent, that may complex with a detectable label, such as a metal, photosensitizing agent, etc. Chelating groups, for example, include an

iminodicarboxylic group or a polyaminopolycarboxylic group. Chelating groups may be attached to a peptide agent of the invention using the methods generally described in Liu et al., *Bioconjugate Chem.* 12(4):653, 2001; Alter et al., U.S.P.N. 5,753,627; and PCT Publication No. WO 91/01144; both of which 5 are hereby incorporated by reference). An analog of the invention may be complexed, through its attached chelating agent, to a detectable label, thereby resulting in an analog that is indirectly labeled. Similarly, cytotoxic or therapeutic agents, may also be attached via a chelating group to a peptide agent of the invention.

10 By "biologically active peptide" is meant any naturally occurring, modified, or synthetic peptide that is involved in a biological process or function. Examples of biologically active peptides include, but are not limited to: hormones, growth factors, neurotransmitters, antigens, antibodies, or fragments thereof, etc. By "peptide" is meant any polypeptide, peptide 15 (including cyclic or branched peptides), or protein comprising two or more amino acids joined to each other by peptide bonds or modified peptide bonds. "Peptide" refers to both short chains, commonly referred to as peptides, oligopeptides, or oligomers, and to longer chains, up to about 100 residues in length. Peptides may contain amino acids other than the 20 gene-encoded 20 amino acids, and linkages other than peptide bonds. "Peptides" include amino acid sequences modified either by natural processes, or by chemical modification techniques which are well known in the art. Modifications may occur anywhere in a polypeptide, including the peptide backbone, the amino acid side-chains, and the amino or carboxyl termini.

25 The notations used herein for the peptide amino acid residues are those abbreviations commonly used in the art. The less common abbreviations Abu, Ava, β -Ala, hSer, Nle, Nva, Pal, Sar, Dab, and Dap stand for 2-amino-butyric acid, amino valeric acid, beta-aminopropionic acid, homoserine, norleucine, norvaline, (2,3, or 4) 3-pyridyl-Ala, 1,4-diaminobutyric acid, sarcosine, and 30 1,3-diaminopropionic acid, respectively. In all aspects of the invention, it is

noted that when amino acids are not designated as either D- or L-amino acids, the amino acid is either an L-amino acid or could be either a D- or L-amino acid.

By "analog" is meant a molecule that differs from, but is structurally, 5 functionally, and/or chemically related to the reference molecule. The analog may retain the essential properties, functions, or structures of the reference molecule. Most preferably, the analog retains at least one biological function of the reference molecule. Generally, differences are limited so that the structure or sequence of the reference molecule and the analog are similar 10 overall. A peptide analog and its reference peptide may differ in amino acid sequence by one or more substitutions, additions, and/or deletions, in any combination. A substituted or inserted amino acid residue may or may not be one encoded by the genetic code. An analog of a peptide or polypeptide may be naturally occurring such as an allelic variant, or it may be a variant that is not 15 known to occur naturally. Non-naturally occurring analogs of peptides may be made by direct synthesis, by modification, or by mutagenesis techniques.

By "somatostatin peptide" is meant a somatostatin analog having at least 20 one biological activity of native somatostatin; preferably, this activity is the ability to specifically bind to a somatostatin receptor on a somatostatin receptor-bearing cell. Many such analogs having biological activity are known and have been described, for example, in Hornik *et al.*, U.S.P.N. 5,770,687; Coy *et al.*, U.S.P.N. 5,708,135; Hoeger *et al.*, U.S.P.N. 5,750,499; McBride *et al.*, U.S.P.N. 5,620,675; Coy *et al.*, U.S.P.N. 5,633,263; Coy *et al.*, U.S.P.N. 5,597,894; Taylor *et al.*, U.S.P.N. 5,073,541; Coy *et al.*, U.S.P.N. 4,904,642; 25 Dean, U.S.P.N. 6,017,509; Hoffman *et al.*, WO 98/47524; and A. E. Bogden, U.S.P.N. 5,411,943, each of which is hereby incorporated by reference.

The term "bombesin peptide" encompasses bombesin peptide analogs 30 having at least one biological activity of native bombesin; preferably, this activity is the ability to specifically bind to one or all of the three known bombesin receptor subtypes on a bombesin receptor-bearing cell. Bombesin

analog include, but are not limited to, peptides selected from the group containing the octapeptide G-Trp-H-I-His-J-K-NHV, wherein G is Gln, Asn, Nle, or Nva; H is Ava, Gly, Leu, Val, Ile, Nle, or Nva; I is β -Ala, 4-aminobutyric acid, Gly, Ala, D-Ala, N-Me-Ala, or N-Me-D-Ala; J is Phe, Tyr, 5 4-Cloro-Phe, 4-Fluoro-Phe, 4-Bromo-Phe, 4-NO₂-Phe, Ala, Gly, Leu, Val, Ile, Nle, or Nva; K is Met, Phe, Tyr, 4-Cloro-Phe, 4-Fluoro-Phe, 4-Bromo-Phe, 4-NO₂-Phe, Ala, Gly, Leu, Val, Ile, Nle, or Nva; and N represents an amide or a N-alkylamide and V is H or a lower alkylamide.

By "alkyl" is meant an aliphatic branched or straight chain hydrocarbon group. An alkyl is optionally substituted with one or more substituents which may be the same or different. By "lower alkyl" is meant a branched or straight chain alkyl group having less than 11 carbon atoms, preferably a C₁-C₈ alkyl. By "lower alkylamide" is meant a lower alkyl group as described above substituted with one or more amide-containing groups.

15 By "hydrophilic biodistribution" is meant the affinity of the peptide agents of the invention for the bodily fluids of a subject administered the peptide agents (e.g., blood, cerebrospinal fluid, urine, or other bodily fluids), such that the peptide agents distribute throughout the body of the subject, but are rapidly secreted in the urine via the kidney, while avoiding uptake by 20 peripheral organs such as liver, gall bladder, and kidney proximal tubules.

By "hydrophilic polymer" is meant a naturally occurring or synthetic water-soluble polymer optionally modified that alters the biodistribution of a peptide agent of the invention. Examples of such polymers include, but are not limited to poly(ethylene glycol) (PEG), poly(vinyl alcohol) (PVA), dextran, 25 hydroxyethyl starch, gelatin, PVP, PHPMA, poly α , β [N(2-hydroxyethyl)]D,L aspartamide (PHEA), polysuccinamide (PSI), etc. These polymers may be modified by, for example, succinylation (negative charge), partial hydrolysis of PSI (carboxylic groups), or reaction with compounds to add amino- or carboxyl- containing groups, etc. Such optional modifications may increase or 30 change the polymer's hydrophilicity or enable coupling to the peptide or

cytotoxic, therapeutic, or chelating segments of a peptide agent of the invention. Such polymers and modifications are known in the art and are described in, for example, Yamoaka et al., *J. Pharmacol. Sci.* 83:601-606, 1994; Rypacek et al., *Pflugers Arch.* 392:211-217, 1982; Yamoaka et al., *J. Pharm. Pharmacol.* 47:479-486, 1995, Francesco, *Bioconjugate Chemistry* 9(4):418-450, 1998, Duncan and Spreafico, *Clin. Pharmacokinet.* 27(4):290-306, 1994, which are hereby incorporated by reference.

Other features and advantages of the invention will be apparent from the following detailed description thereof, and from the claims.

10

Brief Description of the Drawings

FIG 1. is a graph showing the inhibitory effects of a typical methotrexate-somatostatin conjugate on proliferation of neuroblastoma IMR-32 cells compared to non-conjugated methotrexate and non-conjugated 15 somatostatin analog segment. The equivalent potencies of methotrexate-peptide conjugate and methotrexate alone in this *in vitro* system are readily apparent. Control peptide JF-08-89 is D-Ser-D-Ser-D-Ser-D-Ser-D-Ser-Nle-D-Tyr-D-Ser-cyclo[Cys-Phe-D-Trp-Lys-Thr-Cys]-Thr-NH₂ (SEQ ID NO: 10).

FIG. 2. is a graph displaying the biodistribution of hydrophilic peptide 20 agents of the invention. Two ¹²⁵I-labeled, hydrophilic somatostatin analogs are depicted. Note the lack of accumulation of radioactivity in normal tissue including liver, and the rapid and high efficiency elimination of the labeled peptide agents in urine and feces. Peptide JF-08-73 is ¹²⁵I-succinyl-D-Asp-D-Ser-D-Ser-D-Ser-D-Ser-D-Asp-D-Ser-D-Ser-D-Ser-D-Ser-Lys- 25 D-Tyr-D-Tyr-cyclo[Cys-Phe-D-Trp-Lys-Thr-Cys]-Thr-NH₂ (SEQ ID NO: 11). Peptide JF-08-53 is ¹²⁵I-D-Ser-D-Ser-D-Ser-D-Ser-D-Lys-D-Tyr-D-Ser-cyclo[Cys-Phe-D-Trp-Lys-Thr-Cys]-Thr-NH₂ (SEQ ID NO: 12).

Detailed Description

A family of peptide agents has been developed that utilizes a novel, universal linking sequence. The linking sequence enables conjugation of therapeutic agents, cytotoxic agents, chelating groups, or detectable labels to 5 biologically active peptides without substantial loss of biological potency of the targeting segment of the peptide agent. These peptide agents or analogs may additionally include a hydrophilic biodistribution enhancing segment in another aspect of the invention, increasing potency of treatment by targeting the analogs away from the liver towards rapid elimination via the kidney.

10 A peptide agent of the invention is of the general formula:

X-Y-Z-Q (Formula I)

wherein X is optionally selected from cytotoxic agents, a therapeutic agents, detectable labels or chelating groups; Y is an hydrophilic element; Q is a biologically active peptide such as somatostatin or bombesin, and Z is a linking 15 peptide that, when bonded to Q at the N-terminus or to a compatible side-chain amino group of Q, preserves at least 50% of the biological activity of Q. An example of a substitution of Q is position 6 of the GnRH peptides. This position is very tolerant of substitution of, for instance; D-Lys, the epsilon amino group of which could be used for the described attachment of cytotoxic 20 or radioactive groups with retention of receptor affinity. Z has the formula: A-B-C-E-F where A is D-Lys, D-Tyr, D-Ser, or L-Ser, or is deleted; B is D-Lys or D-Tyr, or deleted; C is Lys, Ser, hSer, Nle, Abu, Nva, (2,3, or 4) 3-pyridyl-Ala 9 (Pal), Orn, Dab, Dap, 4-NH₂-Phe, D-4-OH-Pro, or L-4-OH-Pro, or is deleted; E is D-Lys, D-Tyr, D-Ser, D-OH-Pro, L-4-OH-Pro, D-Asn, L-Asn, D- 25 Asp, L-Asp, D-Glu, L-Glu, D-Gln, or L-Gln; and F is D-Lys, D-Tyr, D-Ser, D-OH-Pro, L-4-OH-Pro, 3-iodo-D-Tyr, 3-5 diido-D-Tyr, 3-astatine-D-Tyr, 3-5 astatine-D-Tyr, 3-bromo-D-Tyr, 3-5 dibromo-D-Tyr, D-Asn, L-Asn, D-Asp, L-Asp, D-Glu, L-Glu, D-Gln, or L-Gln; provided that when A, B, C, and E are Tyr, Tyr, Lys, and Tyr, respectively, F is not Lys; and when A, B, C, and E are

Lys, Tyr, Lys, and Tyr, respectively, E is not Tyr or Lys; and when A and B are deleted, and C and E are Lys and Tyr, respectively, F is not Tyr or Lys. The Z peptide linker may thus be 2, 3, 4, or 5 residues in length.

Included among the peptide agents synthesized thus far are the
5 following:

JF-07-100

MTXCOO-CH₂CO-D-Lys-D-Tyr-Lys-D-Tyr-D-Lys-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-NH₂ (SEQ ID NO: 1)

10 JF-08-87A

MTXCOO-CH₂CO-(D-Ser)₅-Lys-D-Tyr-D-Tyr-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-NH₂ (SEQ ID NO: 2)

JF-09-35

15 MTXCOONH-Leu-Ala-Leu-Ala-(D-Ser)₅-Lys-D-Tyr-D-Ser-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-NH₂ (SEQ ID NO: 3)

MTXCOO-CH₂CO-(D-Ser)₅-Lys-D-Tyr-D-Tyr-cyclo[Cys-Phe-D-Trp-Lys-Thr-Cys]-Thr-NH₂ (SEQ ID NO: 4)

20

MTXCOO-CH₂CO-(D-Ser-Ser)₄-Tyr-D-Ser-Gln-Trp-Ala-Val-β-Ala-His-Phe-Nle -NH₂ (SEQ ID NO: 5)

JF-09-73

25 Thiocolchicine-thioether-Lys-(D-Ser)₁₀-Nle-D-Tyr-D-Ser-cyclo[Cys-Phe-D-Trp-Lys-Thr-Cys]-Thr-NH₂ (SEQ ID NO: 6)

JF-09-93

30 Camptothecin-carbonyl-Sar-D-Ser-Nle-D-Tyr-D-Ser-cyclo[Cys-Phe-D-Trp-Lys-Thr-Cys]-Thr-NH₂ (SEQ ID NO: 7)

JF-09-95

Camptothecin-carbonyl-Pro-D-Ser-Nle-D-Tyr-D-Ser-cyclo[Cys-Phe-D-Trp-Lys-Thr-Cys]-Thr- NH₂ (SEQ ID NO: 8)

5 JF-09-99

Camptothecin-carbonyl-Hydroxyproline-D-Ser-Nle-D-Tyr-D-Ser-cyclo[Cys-Phe-D-Trp-Lys-Thr-Cys]-Thr- NH₂ (SEQ ID NO: 9)

Additional sequences of peptide agents of the invention are found in Table 1.

10 The present invention circumvents the accumulation of toxic peptide analog in tissues, through inclusion of both the Z linker, containing hydrophilic resides, and optional inclusion of an elongated hydrophilic linker. These linkers promote rapid elimination of intact, non-bound peptide agents through the kidneys. Polyethylene glycol (PEG), α , β -poly(N-hydroxyethyl)-DL-aspartamide (PHEA), and polyvinylalcohol (PVA) groups contained in this

15 invention are also known to be excellent promoters of rapid renal secretion which is correlated generally with lower potential drug toxicities (Yamoaka et al., *J. Pharmacol. Sci.*, 83:601-606, 1994; Rypacek et al., *Pflugers Arch.*, 392:211-217, 1982; Yamoaka et al., *J. Pharm. Pharmacol.*, 47:479-486, 1995).

20 We posit that these groups also promote lowered toxicity emanating from bioavailable, non-internalized conjugates.

The peptide agents of the invention include a universal linking sequence designed to preserve full biological potency of the peptide analogs when conjugated to a cytotoxic agent. A peptide analog of the invention has a

25 biological potency that is preferably greater than or equal to the parent peptide analog from which it is derived, with a specificity that is greater, lesser, or equivalent to the parent peptide's target specificity. For example, a somatostatin analog may bind to more somatostatin receptor subtypes than naturally occurring somatostatin, or it may bind to a particular receptor

30 subtype. Some analogs of the invention contain D-isomers of amino acids or

analogs thereof, facilitating stable coupling of cytotoxic agents while retaining high receptor affinity and biological potency of the peptide analog. Preferably a cytotoxic agent will be coupled via a linkage likely to encourage release of free cytotoxic agent intracellularly.

5 Additionally, because the somatostatin analogs of the invention are variously hydrophilic, they are water-soluble and, thus, have enhanced use as compared to previous hydrophobic analogs. The hydrophilic analogs described herein are soluble in blood, cerebrospinal fluid, and other bodily fluids, as well as in urine, facilitating excretion by the kidneys. This hydrophilic character
10 facilitates the delivery of the analogs of the invention to almost every area of the body. The invention also discloses specific hydrophilic elements for incorporation into peptide analogs, allowing modulation of the analog's hydrophilicity to adjust for the chemical and structural nature of the various conjugated cytotoxic agents.

15 Somatostatin agonist analogs are rapidly internalized after binding to their receptors (see Lukinius et al, *Acta Onc.*, 38:383-387, 1999) and can thus be used as vectors for targeting various therapeutic agents – such as traditional tumor cytotoxic agents. It is possible that the specificity of such anti-tumor agents can be drastically improved since many tumor types heavily overexpress
20 somatostatin type 2 receptors. In this manner, we propose that the toxic side effects associated with all conjugatable cytotoxic agents can be usefully lowered as long as a potent hybrid molecule can be designed which retains very high affinity for somatostatin receptors.

25 The invention features the use of a linking sequence, referred to as Z in formula I, which we have found enables many long N-terminal amino acid sequences and large molecules to be conjugated to the N-terminus of the somatostatin analogs with full retention of agonist potency. This linking sequence can be used to add longer, enzymatically stable (via use of D-amino acids), hydrophilic peptide sequences (the Y element of formula I) designed to
30 target the resulting conjugates away from the liver towards rapid elimination

via the kidney. Alternatively, a hydrophilic polymer may be linked to the peptide analog via the Z linker. This promotes lowered toxicity emanating from non-internalized conjugates.

The peptide analog also includes an element, denoted as Y in formula I, 5 which may be optimized to facilitate biodistribution of the particular peptide agent conjugated to a cytotoxic or therapeutic group, or a chelating group or detectable label. The Y linker may be a peptide or a polymer such as PEG or PVA. If Y is a hydrophilic peptide, Y may be 1 to 50 amino acids in length, or more preferably 3 to 15 residues in length. For example, Y may be 3, 4, 5, 6, 7, 10 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 amino acid residues in length. When a peptide, Y may contain charged or non-polar amino acids, their analogs or derivatives that are naturally occurring, synthetic or modified.

The linking sequences of the invention are used to conjugate a biologically active peptide referred to as Q in formula I, to various therapeutic 15 or diagnostic agents, referred to as X in formula I. The component X of formula I can be any known cytotoxic or therapeutic moiety, e.g., Antineoplastic agents such as: Acivicin; Aclarubicin; Acodazole Hydrochloride; Acronine; Adozelesin; Adriamycin; Aldesleukin; Altretamine; Ambomycin; A. metantrone Acetate; Aminoglutethimide; Amsacrine; 20 Anastrozole; Anthramycin; Asparaginase; Asperlin; Azacitidine; Azetepa; Azotomycin; Batimastat; Benzodepa; Bicalutamide; Bisantrene Hydrochloride; Bisnafide Dimesylate; Bizelesin; Bleomycin Sulfate; Brequinar Sodium; Bropirimine; Busulfan; Cactinomycin; Calusterone; Camptothecin; 25 Caracemide; Carbetimer; Carboplatin; Carmustine; Carubicin Hydrochloride; Carzelesin; Cedefingol; Chlorambucil; Cirolemycin; Cisplatin; Cladribine; Combretestatin A-4; Crisnatol Mesylate; Cyclophosphamide; Cytarabine; Dacarbazine; DACA (N- [2- (Dimethyl-amino) ethyl] acridine-4-carboxamide); 30 Dactinomycin; Daunorubicin Hydrochloride; Daunomycin; Decitabine; Dexormaplatin; Dezaguanine; Dezaguanine Mesylate; Diaziquone; Docetaxel; Dolasatins; Doxorubicin; Doxorubicin Hydrochloride; Droloxifene;

Droloxifene Citrate; Dromostanolone Propionate; Duazomycin; Edatrexate;
Eflornithine Hydrochloride; Ellipticine; Elsamitucin; Enloplatin; Enpromate;
Epipropidine; Epirubicin Hydrochloride; Erbulozole; Esorubicin
Hydrochloride; Estramustine; Estramustine Phosphate Sodium; Etanidazole;
5 Ethiodized Oil I 131; Etoposide; Etoposide Phosphate; Etoprine; Fadrozole
Hydrochloride; Fazarabine; Fenretinide; Floxuridine; Fludarabine Phosphate;
Fluorouracil; 5-FdUMP; Flurocitabine; Fosquidone; Fostriecin Sodium;
Gemcitabine; Gemcitabine Hydrochloride; Gold Au 198; Homocamptothecin;
Hydroxyurea; Idarubicin Hydrochloride; Ifosfamide; Ilmofosine; Interferon
10 Alfa-2a; Interferon Alfa-2b; Interferon Alfa-n1; Interferon Alfa-n3; Interferon
Beta-I a; Interferon Gamma-I b; Iproplatin; Irinotecan Hydrochloride;
Lanreotide Acetate; Letrozole; Leuprolide Acetate; Liarozole Hydrochloride;
Lometrexol Sodium; Lomustine; Losoxantrone Hydrochloride; Masoprocol;
Maytansine; Mechlorethamine Hydrochloride; Megestrol Acetate;
15 Melengestrol Acetate; Melphalan; Menogaril; Mercaptopurine; Methotrexate;
Methotrexate Sodium; Metoprine; Meturedepa; Mitindomide; Mitocarcin;
Mitocromin; Mitogillin; Mitomalcin; Mitomycin; Mitosper; Mitotane;
Mitoxantrone Hydrochloride; Mycophenolic Acid; Nocodazole;
Nogalamycin; Ormaplatin; Oxisuran; Paclitaxel; Pegaspargase; Peliomycin;
20 Pentamustine; PeplocinSulfate; Perfosfamide; Pipobroman; Piposulfan;
Piroxantrone Hydrochloride; Plicamycin; Plomestane; Porfimer Sodium;
Porfiromycin; Prednimustine; Procarbazine Hydrochloride; Puromycin;
Puromycin Hydrochloride; Pyrazofurin; Rhizoxin; Rhizoxin D; Riboprine;
Rogletimide; Safingol; Safingol Hydrochloride; Semustine; Simtrazene;
25 Sparfosate Sodium; Sparsomycin; Spirogermanium Hydrochloride;
Spiromustine; Spiroplatin; Streptonigrin; Streptozocin; Strontium Chloride Sr
89; Sulofenur; Talisomycin; Taxane; Taxoid; Tecogalan Sodium; Tegafur;
Teloxantrone Hydrochloride; Temoporfin; Teniposide; Teroxirone;
Testolactone; Thiamiprime; Thioguanine; Thiotepa; Thymitaq; Tiazofurin;
30 Tiramazamine; Tomudex; TOP53; Topotecan Hydrochloride; Toremifene

Citrate; Trestolone Acetate; Triciribine Phosphate; Trimetrexate; Trimetrexate Glucuronate; Triptorelin; Tubulozole Hydrochloride; Uracil Mustard; Uredepa; Vapreotide; Verteporfin; Vinblastine; Vinblastine Sulfate; Vincristine; Vincristine Sulfate; Vindesine; Vindesine Sulfate; Vinepidine Sulfate;

5 Vinglycinate Sulfate; Vinleurosine Sulfate; Vinorelbine Tartrate; Vinrosidine Sulfate; Vinzolidine Sulfate; Vorozole; Zeniplatin; Zinostatin; Zorubicin Hydrochloride; 2-Chlorodeoxyadenosine; 2' Deoxyformycin; 9-aminocamptothecin; raltitrexed; N-propargyl-5,8-dideazafolic acid; 2chloro-2'-arabino-fluoro-2'-deoxyadenosine; 2-chloro-2'-deoxyadenosine; anisomycin;

10 trichostatin A; hPRL-G129R; CEP-751; linomide; sulfur mustard; nitrogen mustard (mechlор ethamine); cyclophosphamide; melphalan; chlorambucil; ifosfamide; busulfan; N-methyl-Nnitrosourea (MNU); N, N'-Bis (2-chloroethyl)-N-nitrosourea (BCNU); N- (2-chloroethyl)-N' cyclohexyl-N-nitrosourea (CCNU); N- (2-chloroethyl)-N'- (trans-4-methylcyclohexyl-N-nitrosourea (MeCCNU); N- (2-chloroethyl)-N'- (diethyl) ethylphosphonate-N-nitrosourea (fotemustine); streptozotocin; diacarbazine (DTIC); mitozolomide; temozolomide; thiotepa; mitomycin C; AZQ; adozelesin; Cisplatin; Carboplatin; Ormaplatin; Oxaliplatin; C1-973; DWA 2114R; JM216; JM335; Bis (platinum); tomudex; azacitidine; cytarabine; gemcitabine; 6-

15 20 Mercaptopurine; 6-Thioguanine; Hypoxanthine; teniposide 9-amino camptothecin; Topotecan; CPT-11; Doxorubicin; Daunomycin; Epirubicin; darubicin; mitoxantrone; losoxantrone; Dactinomycin (Actinomycin D); amsacrine; pyrazoloacridine; all-trans retinol; 14-hydroxy-retro-retinol; all-trans retinoic acid; N- (4- Hydroxyphenyl) retinamide; 13-cis retinoic acid; 3-

25 Methyl TTNEB; 9-cis retinoic acid; fludarabine (2-F-ara-AMP); or 2-chlorodeoxyadenosine (2-Cda).

Other anti-neoplastic compounds include, but are not limited to, 20-pi-1,25 dihydroxyvitamin D3; 5-ethynyluracil; abiraterone; aclarubicin; acylfulvene; adecyepenol; adozelesin; aldesleukin; all TK antagonists;

30 altretamine; ambamustine; amidox; amifostine; aminolevulinic acid; amrubicin;

amsacrine; anagrelide; anastrozole; andrographolide; angiogenesis inhibitors; antagonist D; antagonist G; antarelix; anti-dorsalizing morphogenetic protein-1; antiandrogen, prostatic carcinoma; antiestrogen; antineoplaston; antisense oligonucleotides; aphidicolin glycinate; apoptosis gene modulators; apoptosis regulators; apurinic acid; ara-CDP-DL-PTBA; argininedeaminase; asulacrine; atamestane; atrimustine; axinastatin 1; axinastatin 2; axinastatin 3; azasetron; azatoxin; azatyrosine; baccatin III derivatives; balanol; batimastat; BCR/ABL antagonists; benzochlorins; benzoylstaurosporine; beta lactam derivatives; beta-alethine; betaclamycin B; betulinic acid; bFGF inhibitor; bicalutamide; bisantrene; bisaziridinylspermine; bisnafide; bistratene A; bizelesin; breflate; bleomycin A2; bleomycin B2; bropirimine; budotitane; buthionine sulfoximine; calcipotriol; calphostin C; camptothecin derivatives (e.g., 10-hydroxy-camptothecin); canarypox IL-2; capecitabine; carboxamide-amino-triazole; carboxyamidotriazole; CaRest M3; CARN 700; cartilage derived inhibitor; carzelesin; casein kinase inhibitors (ICOS); castanospermine; cecropin B; cetrorelix; chlorins; chloroquinoxaline sulfonamide; cicaprost; cis-porphyrin; cladribine; clomifene analogues; clotrimazole; collismycin A ; collismycin B; combretastatin A4; combretastatin analogue; conagenin; crambescidin 816 ; crisnatol; cryptophycin 8; cryptophycin A derivatives; curacin A; cyclopentanthraquinones; cycloplatam; cypemycin; cytarabine ocfosfate; cytolytic factor; cytostatin; dacliximab; decitabine; dehydrodidemnin B; 2'deoxycoformycin (DCF); deslorelin; dexifosfamide; dexrazoxane; dexverapamil; diaziquone; didemnin B; didox; diethylnorspermine; dihydro-5-azacytidine; dihydrotaxol, 9- ; dioxamycin; diphenyl spiromustine; discodermolide; docosanol; dolasetron; doxifluridine; droloxifene; dronabinol; duocarmycin SA; ebselen; ecomustine; edelfosine; edrecolomab; eflornithine; elemene; emitefur; epirubicin; epothilones (A, R = H; B, R = Me); epithilones; epristeride; estramustine analogue; estrogen agonists; estrogen antagonists; etanidazole; etoposide; etoposide 4'-phosphate (etopofos); exemestane; fadrozole; fazarabine; fenretinide; filgrastim; finasteride; flavopiridol;

flezelastine; fluasterone; fludarabine; fluorodaunorubicin hydrochloride; forfenimex; formestane; fostriecin; fotemustine; gadolinium texaphyrin; gallium nitrate; galocitabine; ganirelix; gelatinase inhibitors; gemcitabine; glutathione inhibitors; hepsulfam; heregulin; hexamethylene bisacetamide;

5 homoharringtonine (HHT); hypericin; ibandronic acid; idarubicin; idoxifene; idramantone; ilmofosine; ilomastat; imidazoacridones; imiquimod; immunostimulant peptides; insulin-like growth factor-1 receptor inhibitor; interferon agonists; interferons; interleukins; iobenguane; iododoxorubicin; ipomeanol, 4-; irinotecan; iroplact; 10sogladine; isobengazole;

10 isohomohalicondrin B; itasetron; jasplakinolide; kahalalide F; lamellarin-N triacetate; lanreotide; leinamycin; lenograstim; lentinan sulfate; leptolstatin; letrozole; leukemia inhibiting factor; leukocyte alpha interferon; leuprolide + estrogen + progesterone; leuprorelin; levamisole; liarozole; linear polyamine analogue; lipophilic disaccharide peptide; lipophilic platinum compounds;

15 lissoclinamide 7; lobaplatin; lombricine; lometrexol; lonidamine; losoxantrone; lovastatin; loxoribine; lurtotecan; lutetium texaphyrin; lysofylline; lytic peptides; maytansine; mannostatin A; marimastat; masoprolol; maspin; matrilysin inhibitors; matrix metalloproteinase inhibitors; menogaril; mnerbarone; meterelin; methioninase; metoclopramide; MIF inhibitor;

20 ifepristone; miltefosine; mirimostim; mismatched double stranded RNA; mithracin; mitoguazone; mitolactol; mitomycin analogues; mitonafide; mitotoxin fibroblast growth factor-saporin; mitoxantrone; mofarotene; molgramostim; monoclonal antibody, human chorionic gonadotrophin; monophosphoryl lipid A + myobacterium cell wall sk; mopidamol; multiple

25 drug resistance gene inhibitor; multiple tumor suppressor 1-based therapy; mustard anticancer agent; mycaperoxide B; mycobacterial cell wall extract; myriaporone; N-acetyldinaline; N-substituted benzamides; nafarelin; nagrestip; naloxone + pentazocine; napavin; naphterpin; nartograstim; nedaplatin; nemorubicin; neridronic acid; neutral endopeptidase; nilutamide; nisamycin;

30 nitric oxide modulators; nitroxide antioxidant; nitrullyn; 06-benzylguanine;

octreotide; okicenone; oligonucleotides; onapristone; ondansetron; ondansetron; oracin; oral cytokine inducer; ormaplatin; osaterone; oxaliplatin; oxaunomycin; paclitaxel analogues; paclitaxel derivatives; palauamine; palmitoylrhizoxin; pamidronic acid; panaxytriol; panomifene; parabactin; 5 pazelliptine; pegaspargase; peldesine; pentosan polysulfate sodium; pentostatin; pentozole; perflubron; perfosfamide; perillyl alcohol; phenazinomycin; phenylacetate; phosphatase inhibitors; picibanil; pilocarpine hydrochloride; pirarubicin; piritrexim; placetin A; placetin B; plasminogen activator inhibitor; platinum complex; platinum compounds; platinum-triamine 10 complex; podophyllotoxin; porfimer sodium; porfiromycin; propyl bis-acridone; prostaglandin J2; proteasome inhibitors; protein A-based immune modulator; protein kinase C inhibitor; protein kinase C inhibitors, microalgal; protein tyrosine phosphatase inhibitors; purine nucleoside phosphorylase inhibitors; purpurins; pyrazoloacridine; pyridoxylated hemoglobin 15 polyoxyethylene conjugate; raf antagonists; raltitrexed; ramosetron; ras farnesyl protein transferase inhibitors; ras inhibitors; ras-GAP inhibitor; retelliptine demethylated; rhenium Re 186 etidronate; rhizoxin; ribozymes; RII retinamide; rogletimide; rohitukine; romurtide; roquinimex; rubiginone B 1; ruboxyl; safingol; saintopin; SarCNU; sarcophytol A; sargramostim; Sdi 1 20 mimetics; semustine; senescence derived inhibitor 1; sense oligonucleotides; signal transduction inhibitors; signal transduction modulators; single chain antigen binding protein; sizofiran; sobuzoxane; sodium borocaptate; sodium phenylacetate; solverol; somatomedin binding protein; sonermin; sparfosic acid; spicamycin D; spiromustine; splenopentin; spongistatin 1; squalamine; 25 stem cell inhibitor; stem-cell division inhibitors; stipiamide; stromelysin inhibitors; sulfinosine; superactive vasoactive intestinal peptide antagonist; suradista; suramin; swainsonine; synthetic glycosaminoglycans; tallimustine; tamoxifen methiodide; tauromustine; tazarotene; tecogalan sodium; tegafur; tellurapyrylium; telomerase inhibitors; temoporfin; temozolomide; teniposide; 30 tetrachlorodecaoxide; tetrazomine; thaliblastine; thalidomide; thiocoraline;

thrombopoietin; thrombopoietin mimetic; thymalfasin; thymopoietin receptor agonist; thymotrinan; thyroid stimulating hormone; tin ethyl etiopurpurin; tirapazamine; titanocene dichloride; topotecan; topsentin; toremifene; totipotent stem cell factor; translation inhibitors; tretinoin; triacetyluridine; triciribine; 5 trimetrexate; triptorelin; tropisetron; turosteride; tyrosine kinase inhibitors; tyrophostins; UBC inhibitors; ubenimex; urogenital sinus-derived growth inhibitory factor; urokinase receptor antagonists; vapreotide; variolin B; vector system, erythrocyte gene therapy; velaresol; veramine; verdins; verteporfin; vinorelbine; vinxaltine; vitaxin; vorozole; zanoterone; zeniplatin; zilascorb; and 10 zinostatin stimalamer.

X may also be an antiproliferative agent, for example piritrexim isothionate. Alternatively, X may be an antiprostatic hypertrophy agent such as, for example, sitogluside, a benign prostatic hyperplasia therapy agent such as, for example, tamsulosin hydrochloride, or a prostate growth inhibitor such 15 as, for example, pentomone.

X can also be a radioactive agent, including, but not limited to:

Fibrinogen ¹²⁵I; Fludeoxyglucose ¹⁸F; Fluorodopa ¹⁸F; Insulin ¹²⁵I; Insulin ¹³¹I; lobenguane ¹²³I; Iodipamide Sodium ¹³¹I; Iodoantipyrine ¹³¹I; Iodocholesterol ¹³¹I; Iodohippurate Sodium ¹²³I; Iodohippurate Sodium ¹²⁵I; Iodohippurate 20 Sodium ¹³¹I; Iodopyracet ¹²⁵I; Iodopyracet ¹³¹I; lofetamine Hydrochloride ¹²³I; Iomethin ¹²⁵I; Iomethin ¹³¹I; Iothalamate Sodium ¹²⁵I; Iothalamate Sodium ¹³¹I; tyrosine ¹³¹I; Liothyronine ¹²⁵I; Liothyronine ¹³¹I; Merisoprol Acetate ¹⁹⁷Hg; Merisoprol Acetate ²⁰³Hg; Merisoprol ¹⁹⁷Hg; Selenomethionine ⁷⁵Se; 25 Technetium ^{99m}Tc Antimony Trisulfide Colloid; Technetium ^{99m}Tc Bicisate; Technetium ^{99m}Tc Disofenin; Technetium ^{99m}Tc Etidronate; Technetium ^{99m}Tc Exametazime; Technetium ^{99m}Tc Furifosmin; Technetium ^{99m}Tc Gluceptate; 30 Technetium ^{99m}Tc Lidofenin; Technetium ^{99m}Tc Mebrofenin; Technetium ^{99m}Tc Medronate; Technetium ^{99m}Tc Medronate Disodium; Technetium ^{99m}Tc Mertiatide; Technetium ^{99m}Tc Oxidronate; Technetium ^{99m}Tc Pentetate; Technetium ^{99m}Tc Pentetate Calcium Trisodium; Technetium ^{99m}Tc Sestamibi;

Technetium ^{99m}Tc Siboroxime; Technetium ^{99m}Tc; Succimer; Technetium ^{99m}Tc Sulfur Colloid; Technetium ^{99m}Tc Teboroxime; Technetium ^{99m}Tc Tetrofosmin; Technetium ^{99m}Tc Tiatide; Thyroxine ¹²⁵I; Thyroxine ¹³¹I; Tolpovidone ¹³¹I; Triolein ¹²⁵I; or Triolein ¹³¹I.

5 Therapeutic or cytotoxic agents may include, for example, anti-cancer Supplementary Potentiating Agents, including, but not limited to: Tricyclic anti-depressant drugs (e.g., imipramine, desipramine, amitryptyline, clomipramine, trimipramine, doxepin, nortriptyline, protriptyline, amoxapine, and maprotiline); non-tricyclic anti-depressant drugs (e.g., sertraline, 10 trazodone, and citalopram); Ca⁺⁺ antagonists (e.g., verapamil, nifedipine, nitrendipine, and caroverine); Calmodulin inhibitors (e.g., prenylamine, trifluoroperazine, and clomipramine); Amphotericin B; Triparanol analogues (e.g., tamoxifen); antiarrhythmic drugs (e.g., quinidine); antihypertensive drugs (e.g., reserpine); Thiol depleters (e.g., buthionine and sulfoximine) and 15 Multiple Drug Resistance reducing agents such as Cremaphor EL.

The compounds of the invention also can be administered with cytokines such as granulocyte colony stimulating factor. Preferred anticancer agents used in anti-cancer cocktails (e.g., in combination with the agents of the invention) include (some with their MTDs shown in parentheses): gemcitabine 20 (1000 mg/m²); methotrexate (15 gm/m² i.v. + leuco. < 500 mg/m² i.v. w/o leuco); 5-FU (500 mg/m²/day x 5days); FUDR (100 mg/kg x 5 in mice, 0.6 mg/kg/day in human i.a.); FdUMP; Hydroxyurea (35 mg/kg/d in man); Docetaxel (60-100 mg/m²); discodermolide; epothilones; vincristine (1.4 mg/m²); vinblastine (escalating: 3.3-11.1mg/m², or rarely to 18.5 mg/m²); 25 vinorelbine (30 mg/m²/wk); meta-pac; irinotecan (50-150 mg/m², 1 x/wk depending on patient response); SN-38 (-100 times more potent than Irinotecan); 10-OH campto; topotecan (1.5 mg/m²/day in humans, 1 x iv LD₁₀mice=75 mg/m²); etoposide (100 mg/m² in man); adriamycin; flavopiridol; Cis-Pt (100mg/m² in man); carbo-Pt (360 mg/m² in man); 30 bleomycin (20 mg/m²); mitomycin C (20 mg/m²); mithramycin (30 sug/kg);

capecitabine (2.5 g/m² orally); cytarabine (100 mg/m²/day); 2-Cl-2'deoxyadenosine; Fludarabine-P04 (25 mg/m²/day, x 5days); mitoxantrone (12-14 mg/m²); mitozolomide (> 400 mg/m²); Pentostatin; or Tomudex.

X may preferably be an antimetabolic agent, such as methotrexate.

5 Antimetabolites include, but are not limited to, the following compounds and their derivatives: azathioprine, cladribine, cytarabine, dacarbazine, fludarabine phosphate, fluorouracil, gencitabine chlorhydrate, mercaptoperine, methotrexate, mitobronitol, mitotane, proguanil chlorhydrate, pyrimethamine, raltitrexed, trimetrexate glucuronate, urethane, vinblastine sulfate, vincristine
10 sulfate, etc. More preferably, X may be a folic acid-type antimetabolite, a class of agents that includes, for example, methotrexate, proguanil chlorhydrate, pyrimethamine, trimethoprim, or trimetrexate glucuronate, or derivatives of these compounds.

In another embodiment, X may be a member of the anthracycline family
15 of neoplastic agents, including but not limited to aclarubicine chlorhydrate, daunorubicine chlorhydrate, doxorubicine chlorhydrate, epirubicine chlorhydrate, idarubicine chlorhydrate, pirarubicine, or zorubicine chlorhydrate. Furthermore, X may be a camptothecin, or its derivatives or related compounds such as 10, 11 methylenedioxycamptothecin. X may also
20 be selected from the maytansinoid family of compounds, which includes a variety of structurally related compounds. For example, ansamitocin P3, maytansine, 2'-N-demethylmaytanbutine, or maytanbicyclinol are maytansinoids.

The peptide agents of the invention can be modified or labeled to
25 facilitate diagnostic or therapeutic uses. Detectable labels such as a radioactive, fluorescent, heavy metal, or other agents may be bound to the peptide agents of the invention. Single, dual, or multiple labeling of a peptide agent may be advantageous. For example, dual labeling with radioactive iodination of one or more residues combined with the additional coupling of,
30 for example, ⁹⁰Y via a chelating group to amine-containing side or reactive

groups, would allow combination labeling. This may be useful for specialized diagnostic needs such as identification of widely dispersed small neoplastic cell masses.

Peptide analogs of the invention may also be modified, for example, by 5 halogenation of the tyrosine residues of the compound. Halogens include fluorine, chlorine, bromine, iodine, or astatine. Such halogenated peptide agents may be detectably labeled, e.g. if the halogen is a radioisotope such as, for example, ¹⁸F, ⁷⁵Br, ⁷⁷Br, ¹²²I, ¹²³I, ¹²⁴I, ¹²⁵I, ¹²⁹I, ¹³¹I, or ²¹¹At. Halogenated compounds of the invention contain a halogen covalently bound to at least one 10 amino acid, and preferably to D-Tyr residues in each peptide agent molecule. Other suitable detectable modifications include binding of other compounds (e.g., a fluorochrome such as fluorescein) to a lysine residue of the analog, particularly an analog having a linker including lysines.

Radioisotopes for radiolabeling the biological peptide agents of the 15 invention include any radioisotope that can be covalently bound to a residue of the analog. The radioisotopes can be selected from radioisotopes that emit either beta or gamma radiation, or alternatively, the peptide agents can be modified to contain chelating groups that, for example, can be covalently bonded to lysine residue(s) of the analog. The chelating groups can then be 20 modified to contain any of a variety of radioisotopes, such as gallium, indium, technetium, ytterbium, rhenium, or thallium (e.g., ¹²⁵I, ⁶⁷Ga, ¹¹¹In, ^{99m}Tc, ¹⁶⁹Yb, ¹⁸⁶Re).

Where the peptide agent is modified by attachment of a radioisotope, 25 preferable radioisotopes are those having a radioactive half-life corresponding to, or longer than, the biological half-life of the agent used. More preferably, the radioisotope is a radioisotope of a halogen atom (e.g. a radioisotope of fluorine, chlorine, bromine, iodine, and astatine), even more preferably ⁷⁵Br, ⁷⁷Br, ⁷⁶Br, ¹²²I, ¹²³I, ¹²⁴I, ¹²⁵I, ¹²⁹I, ¹³¹I, or ²¹¹At.

Conjugates that include radioactive metals are useful in radiographic 30 imaging or radiotherapy. Preferred radioisotopes also include ^{99m}Tc, ⁵¹Cr,

^{67}Ga , ^{68}Ga , ^{111}In , ^{168}Yb , ^{140}La , ^{90}Y , ^{88}Y , ^{153}Sm , ^{156}Ho , ^{165}Dy , ^{64}Cu , ^{97}Ru , ^{103}Ru , ^{186}Re , ^{188}Re , ^{203}Pb , ^{211}Bi , ^{212}Bi , ^{213}Bi , and ^{214}Bi . The choice of metal is determined based on the desired therapeutic or diagnostic application.

The metal complexes of the invention are useful as diagnostic and/or therapeutic agents. A detectable label may be a metal ion from heavy elements or rare earth ions, such as Gd^{3+} , Fe^{3+} , Mn^{3+} , or Cr^{2+} . Conjugates that include paramagnetic or superparamagnetic metals are useful as diagnostic agents in MRI imaging applications. Paramagnetic metals that may be used in the conjugates include, but are not limited to, chromium (III), manganese (II), iron (II), iron (III), cobalt (II), nickel (II), copper (II), praseodymium (III), neodymium (III), samarium (III), gadolinium (III), terbium (III), dysprosium (III), holmium (III), erbium (III), and ytterbium (III). Preferably, the polymer has a relaxivity of at least 10, 12, 15, or 20 $\text{mM}^{-1} \text{sec}^{-1} \text{Z}^{-1}$, wherein Z is the concentration of paramagnetic metal.

Chelating groups may be used to indirectly couple detectable labels or other molecules to the peptide agents of the invention. Chelating groups may link peptide agents with radiolabels, such as a bifunctional stable chelator may be linked to one or more terminal or internal amino acid reactive groups. They may be linked via an isothiocyanate β -Ala or appropriate non α -amino acid linker which prevents Edman degradation. Examples of chelators known in the art include, for example, the ininocarboxylic and polyaminopolycarboxylic reactive groups, DTPA (N,N-Bis[2-[bis(carboxymethyl)amino]ethyl]glycine), and DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid).

The cytotoxic or therapeutic conjugates of the invention can employ any of the large number of known somatostatin analogs that recognize the somatostatin receptor, e.g., those described in the definitions above. Preferably, the somatostatin analog portion of the conjugate contains between 10 and 18 amino acids, and includes the core sequence: cyclo[Cys-Phe-D-Trp-Lys-Thr-Cys]. Preferably, the C-terminus of the analog is: Thr-NH₂.

Bombesin analogs as disclosed herein may also be conjugated to cytotoxic or therapeutic agents in the peptide agents of the invention.

The somatostatin analog can be coupled directly to the cytotoxic or therapeutic agent using known chemical methods, or the two moieties can be 5 coupled via an indirect linkage. For example, the analog may be attached to a chelating group that is attached to the cytotoxic or therapeutic agent. Chelating groups include, but are not limited to, an iminodicarboxylic group or a polyaminopolycarboxylic group. For general methods, see, e.g., Liu et al., *Bioconjugate Chem.* 12(4):653, 2001; Cheng et al., WO 89/12631; Kieffer et 10 al., WO 93/12112; Albert et al., U.S.P.N. 5,753,627; and WO 91/01144 (each of which are hereby incorporated by reference).

Specific targeting of labeled, therapeutic or cytotoxic agents allows 15 selective destruction of tumors expressing receptors specific for biologically active peptides. For example, the tumors expressing somatostatin receptors includes neoplasms of the lung, breast, prostate, colon, brain, gastrointestinal tract, neuroendocrine axis, liver, kidney, etc. (see Schaer et al., *Int. J. Cancer*, 70:530-537, 1997; Chave et al., *Br. J. Cancer* 82(1):124-130, 2000; Evans et al., *Br. J. Cancer* 75(6):798-803, 1997). Cytotoxic somatostatin peptide 20 analogs may also be specific for tumor vasculatures, or angiogenic blood vessels, such as those which over-express somatostatin receptors (see Denzler and Reubi, *Cancer* 85:188-198, 1999; Gulec et al., *J. Surg. Res.* 97(2):131-137, 2001; Woltering et al., *J. Surg. Res.* 50:245, 1991).

Peptide agents of the invention may be administered to a mammalian 25 subject, such as a human, directly or in combination with any pharmaceutically acceptable carrier or salt known in the art. Pharmaceutically acceptable salts may include non-toxic acid addition salts or metal complexes that are commonly used in the pharmaceutical industry. Examples of acid addition salts include organic acids such as acetic, lactic, pamoic, maleic, citric, malic, ascorbic, succinic, benzoic, palmitic, suberic, salicylic, tartaric, 30 methanesulfonic, toluenesulfonic, or trifluoroacetic acids or the like; polymeric

acids such as tannic acid, carboxymethyl cellulose, or the like; and inorganic acids such as hydrochloric acid, hydrobromic acid, sulfuric acid phosphoric acid, or the like. Metal complexes include zinc, iron, and the like. One exemplary pharmaceutically acceptable carrier is physiological saline. Other 5 physiologically acceptable carriers and their formulations are known to one skilled in the art and described, for example, in Remington's Pharmaceutical Sciences, (18th edition), ed. A. Gennaro, 1990, Mack Publishing Company, Easton, PA.

10 Pharmaceutical formulations of a therapeutically effective amount of a peptide agent of the invention, or pharmaceutically acceptable salt-thereof, can be administered orally, parenterally (e.g., intramuscular, intraperitoneal, intravenous, or subcutaneous injection, inhalation, intradermally, optical drops, or implant), nasally, vaginally, rectally, sublingually, or topically, in admixture with a pharmaceutically acceptable carrier adapted for the route of 15 administration.

Methods well known in the art for making formulations are found, for example, in Remington's Pharmaceutical Sciences (18th edition), ed. A. Gennaro, 1990, Mack Publishing Company, Easton, PA. Compositions 20 intended for oral use may be prepared in solid or liquid forms according to any method known to the art for the manufacture of pharmaceutical compositions. The compositions may optionally contain sweetening, flavoring, coloring, perfuming, and/or preserving agents in order to provide a more palatable preparation. Solid dosage forms for oral administration include capsules, tablets, pills, powders, and granules. In such solid forms, the active compound 25 is admixed with at least one inert pharmaceutically acceptable carrier or excipient. These may include, for example, inert diluents, such as calcium carbonate, sodium carbonate, lactose, sucrose, starch, calcium phosphate, sodium phosphate, or kaolin. Binding agents, buffering agents, and/or lubricating agents (e.g., magnesium stearate) may also be used. Tablets and 30 pills can additionally be prepared with enteric coatings.

Liquid dosage forms for oral administration include pharmaceutically acceptable emulsions, solutions, suspensions, syrups, and soft gelatin capsules. These forms contain inert diluents commonly used in the art, such as water or an oil medium. Besides such inert diluents, compositions can also include 5 adjuvants, such as wetting agents, emulsifying agents, and suspending agents.

Formulations for parenteral administration include sterile aqueous or non- aqueous solutions, suspensions, or emulsions. Examples of suitable vehicles include propylene glycol, polyethylene glycol, vegetable oils, gelatin, hydrogenated naphthalenes, and injectable organic esters, such as ethyl oleate. 10 Such formulations may also contain adjuvants, such as preserving, wetting, emulsifying, and dispersing agents. Biocompatible, biodegradable lactide polymer, lactide/glycolide copolymer, or polyoxyethylene-polyoxypropylene copolymers may be used to control the release of the compounds. Other potentially useful parenteral delivery systems for the polypeptides of the 15 invention include ethylene-vinyl acetate copolymer particles, osmotic pumps, implantable infusion systems, and liposomes.

Liquid formulations can be sterilized by, for example, filtration through a bacteria-retaining filter, by incorporating sterilizing agents into the compositions, or by irradiating or heating the compositions. Alternatively, they 20 can also be manufactured in the form of sterile, solid compositions which can be dissolved in sterile water or some other sterile injectable medium immediately before use.

Compositions for rectal or vaginal administration are preferably 25 suppositories which may contain, in addition to active substances, excipients such as coca butter or a suppository wax. Compositions for nasal or sublingual administration are also prepared with standard excipients known in the art. Formulations for inhalation may contain excipients, for example, lactose, or may be aqueous solutions containing, for example, polyoxyethylene-9-lauryl ether, glycocholate and deoxycholate, or may be oily solutions for 30 administration in the form of nasal drops or spray, or as a gel.

The amount of active ingredient in the compositions of the invention can be varied. One skilled in the art will appreciate that the exact individual dosages may be adjusted somewhat depending upon a variety of factors, including the polypeptide being administered, the time of administration, the 5 route of administration, the nature of the formulation, the rate of excretion, the nature of the subject's conditions, and the age, weight, health, and gender of the patient. In addition, the severity of the condition targeted by the biologically active peptide such as somatostatin or bombesin will also have an impact on the dosage level. Generally, dosage levels of between 0.1 $\mu\text{g}/\text{kg}$ to 10 100 mg/kg of body weight are administered daily as a single dose or divided into multiple doses. Preferably, the general dosage range is between 250 $\mu\text{g}/\text{kg}$ to 5.0 mg/kg of body weight per day. Wide variations in the needed dosage are to be expected in view of the differing efficiencies of the various routes of administration. For instance, oral administration generally would be expected 15 to require higher dosage levels than administration by intravenous injection. Variations in these dosage levels can be adjusted using standard empirical routines for optimization, which are well known in the art. In general, the precise therapeutically effective dosage will be determined by the attending physician in consideration of the above identified factors.

20 The polypeptides of the invention can be administered in a sustained release composition, such as those described in, for example, U.S.P.N. 5,672,659 and U.S.P.N. 5,595,760. The use of immediate or sustained release compositions depends on the type of condition being treated. If the condition consists of an acute or over-acute disorder, a treatment with an immediate 25 release form will be preferred over a prolonged release composition. Alternatively, for preventative or long-term treatments, a sustained released composition will generally be preferred.

30 Polypeptides of the present invention can be prepared in any suitable manner. The polypeptides may be isolated from naturally occurring sources, recombinantly produced, or produced synthetically, or produced by a

combination of these methods. The synthesis of short peptides is well known in the art. See e.g., Stewart et al., Solid Phase Peptide Synthesis (Pierce Chemical Co., 2d ed., 1984). The peptides of the present invention can be synthesized according to standard peptide synthesis methods known in the art 5 and exemplified in Example 1 below.

The present invention is illustrated by the following examples, which are in no way intended to be limiting of the invention.

EXAMPLE 1

10 Preparation of di-cesium salt of (+)-amethopterin (methotrexate).

Methotrexate (0.75 mmole) was dissolved in 200 ml of water to which was added cesium bicarbonate (1.5 mmol). The yellow solution was stirred for 4 hours, evaporated under reduced pressure, and the oily residue dissolved in 15 ethanol. This was evaporated from anhydrous ethanol 3 times to remove residual water.

EXAMPLE 2

Preparation of 2-bromo-acetyl-D-tert-butyl-Ser-D-tert-butyl-Ser-D-tert-butyl-
20 Ser-D-tert-butyl-Ser-D-tert-butyl-Ser-epsilon-tert-butyloxycarbonyl-Lys-D-
tert-butyl-Tyr-D-tert-butyl-Tyr-S-trityl-Cys-Phe-D-Trp-epsilon-tert-
butyloxycarbonyl-Lys-tert-butyl-Thr-S-trityl-Cys-tert-butyl-Thr-Rink-amide-
resin.

25 Rink amide MBHA polystyrene resin (0.25 mmole) [4-(2',4'-
dimethoxyphenyl-Fmoc-(aminomethyl)phenoxyacetamido-norleucyl-
methylbenzhydrylamine resin, 100-200 mesh, Novabiochem, San Diego, CA]
was added to the reaction vessel of a CS136 automatic peptide synthesizer (CS
Bio, Inc., San Carlos, CA) and swollen in DMF for approximately 1 hour. The
30 resin was filtered and an excess of 20% piperidine in DMF was added and

mixed for 2 minutes. The resin was filtered and again an excess amount of 20% piperidine added and mixed for 20 minutes to ensure complete removal of the resin Fmoc group. After deprotection, the resin was washed 4 times with DMF and then the first protected amino acid, Fmoc-Thr(tBut) (0.75 mmol), 5 diisopropylcarbodiimide (DIC) (0.75 mmol), and N-hydroxybenzotriazole monohydrate (HOBt) (0.75 mmol) were all dissolved in DMF and added to the resin which was mixed (1 h) followed by washing 4 times with DMF.

The Fmoc group was again removed by treatment with 20% piperidine/DMF solution and, following the same general coupling procedures, 10 the following amino acids were successively reacted with the growing peptide chain: Fmoc-S-trityl-L-cysteine, Fmoc-O-t-butyl-L-threonine, N^a-Fmoc-N^e-Boc-L-lysine, N^a-Fmoc-Nⁱⁿ-Boc-D-tryptophan, Fmoc-L-phenylalanine, Fmoc-S-trityl-L-cysteine, Fmoc-O-t-butyl-D-tyrosine, Fmoc-O-t-butyl-D-tyrosine, N^a-Fmoc-N^e-Boc-L-lysine, Fmoc-O-t-butyl-D-serine, Fmoc-O-t-butyl-D-serine, 15 Fmoc-O-t-butyl-D-serine, Fmoc-O-t-butyl-D-serine, Fmoc-O-t-butyl-D-serine. After removal of the final Fmoc group, 2-bromoacetic acid was coupled to the N-terminal amino group using the same coupling reagents.

EXAMPLE 3

20 Preparation of methotrexate-acetyl-D-tert-butyl-Ser-D-tert-butyl-Ser-D-tert-butyl-Ser-D-tert-butyl-Ser-D-tert-butyl-Ser-epsilon-tert-butyloxycarbonyl-Lys-D-tert-butyl-Tyr-D-tert-butyl-Tyr-S-trityl-Cys-Phe-D-Trp-epsilon-tert-butyloxycarbonyl-Lys-tert-butyl-Thr-S-trityl-Cys-tert-butyl-Thr-Rink-amide-resin.

25

The di-cesium salt of methotrexate (0.75 mmol) prepared in Example 1 was dissolved in DMSO and added to peptidyl resin (0.25 mmol) prepared in Example 2 in a round bottom flask. The suspension was gently mixed while

heating in a water bath (40°C, 18 h), filtered, and washed with copious amounts of DMF followed by methanol. After a final filtration the derivatized resin was air dried overnight.

EXAMPLE 4

5 Preparation of methotrexate-acetyl-D-Ser-D-Ser-D-Ser-D-Ser-D-Ser-Lys-D-Tyr-D-Tyr-cyclo[Cys-Phe-D-Trp-Lys-Thr-Cys]-Thr-amide.

The methotrexate-peptide resin prepared in Example 3 (0.25 mmol) was placed in a round bottomed flask to which was added 15 ml of a solution of 10 trifluoroacetic acid (TFA) containing water (2.5%), 1,2-ethanedithiol (2.5%), and triisopropylsilane (1%). The suspension was agitated (2 h), filtered, and washed several times with TFA. The TFA was evaporated *in vacuo* and ether added to the resulting oil to give a yellow powder which was then dissolved in 15 60% acetic acid (250 ml). A concentrated solution of iodine in methanol was added dropwise with vigorous stirring until a permanent brown coloration was formed whereupon excess iodine was removed by addition of a small quantity of ascorbic acid.

The solution was reduced to a volume of around 10 ml *in vacuo* and the crude methotrexate peptide purified by preparative reverse phase high pressure 20 liquid chromatography (RP-hplc) on a column (21.4 x 250 mm) of C-18 bonded silica (Dynamax 300, 8 µm). A linear gradient elution system at a flow rat of 20 mL/min was employed: buffer A consisted of 0.1% TFA and buffer B, 0.1% TFA in 80% MeCN; 20% B to 50% B was increased at 1% per min. The separation were monitored at 280nm. The fractions containing the pure 25 product as evidenced by analytical hplc were pooled, concentrated *in vacuo*, and subjected to lyophilization. The peptide was obtained as a fluffy white powder of constant weight by lyophilization from aqueous acetic acid. Correct composition was demonstrated by amino acid analysis of an acid hydrolysate and matrix assisted laser desorption mass spectrometry.

EXAMPLE 5

Preparation of 2-bromo-acetyl-D-tert-butyl-Ser-tert-butyl-Ser-D-tert-butyl-Ser-tert-butyl-Ser-D-tert-butyl-Ser-tert-butyl-Ser-D-tert-butyl-Ser-tert-butyl-Ser-D-tert-butyl-Ser-tert-butyl-Ser-D-tert-butyl-Tyr-D-tert-butyl-Ser-Gln-Trp-Ala-Val- β -Ala-tert-butyloxycarbonyl-

5 His-Phe-Nle-Rink-amide-resin.

Rink amide MBHA polystyrene resin (0.25 mmole) [4-2', 4'-dimethoxyphenyl-Fmoc-(aminomethyl)phenoxyacetamido-norleucyl-methylbenzhydrylamine resin, 100-200 mesh, (Novabiochem, San Diego, CA)]
10 was added to the reaction vessel of a CS136 automatic peptide synthesizer (CS Bio, Inc., San Carlos, CA), and swollen in DMF for approximately 1 hour. The resin was filtered and an excess of 20% piperidine in DMF was added and mixed for 2 minutes. The resin was filtered and again an excess amount of 20% piperidine added and mixed for 20 minutes to ensure complete removal of 15 the resin Fmoc group. After deprotection, the resin was washed 4 times with DMF and then the first protected amino acid, Fmoc-Nle (0.75 mmol), diisopropylcarbodiimide (DIC) (0.75 mmol), and N-hydroxybenzotriazole monohydrate (HOEt) (0.75 mmol) were all dissolved in DMF and added to the resin which was mixed (1 h) followed by washing 4 times with DMF.

20 The Fmoc group was again removed by treatment with 20% piperidine/DMF solution and the remaining amino acid derivatives and 2 bromoacetic acid were coupled as described in Example 2 using the same coupling reagents.

25

EXAMPLE 6

Preparation of methotrexate-acetyl-D-tert-butyl-Ser-tert-butyl-Ser-D-tert-butyl-Ser-tert-butyl-Ser-D-tert-butyl-Ser-tert-butyl-Ser-D-tert-butyl-Ser-D-tert-butyl-Ser-tert-butyl-Ser-D-tert-butyl-Ser-tert-butyl-Tyr-D-tert-butyl-Ser-Gln-Trp-Ala-Val- β -Ala-tert-butyloxycarbonyl-His-Phe-Nle-Rink-amide-resin.

The di-cesium salt of methotrexate (0.75 mmol) prepared in Example 1 was dissolved in DMSO and added to peptidyl resin (0.25 mmol) prepared in Example 5 in a round bottom flask. The suspension was gently mixed while heating in a water bath (40°C, 18 h), filtered, and washed with copious amounts 5 of DMF followed by methanol. After a final filtration the derivatized resin was air dried overnight.

EXAMPLE 7

Methotrexate-acetyl-D-Ser-Ser-D-Ser-Ser-D-Ser-Ser-D-Ser-Tyr-D-Ser-
10 Gln-Trp-Ala-Val-β-Ala-His-Phe-Nle-NH₂.

The methotrexate-peptide resin prepared in Example 6 (0.25 mmol) was placed in a round bottomed flask to which was added 15 ml of a solution of trifluoroacetic acid (TFA) containing water (2.5%), 1,2 ethanedithiol (2.5%), 15 and triisopropylsilane (1%). The suspension was agitated (2 h), filtered, and washed several times with TFA. The TFA was evaporated *in vacuo* and ether was added to the resulting oil to give a yellow powder.

The methotrexate peptide was purified by preparative reverse phase high pressure liquid chromatography (RP-hplc) on a column (21.4x250 mm) of C-18 20 bonded silica (Dynamax 300m 8 μm). A linear gradient elution system at a flow rate of 20 mL/min was employed: buffer A consisted of 0.1% TFA and buffer B, 0.1% TFA in 80% MeCN; 20% B to 50% B was increased at 1%/min. The separation was monitored at 280 nm. The fractions containing the pure 25 product as evidenced by analytical HPLC were pooled, concentrated *in vacuo*, and subjected to lyophilization. The peptide was obtained as a fluffy white powder of constant weight by lyophilization from aqueous acetic acid. Correct composition was demonstrated by amino acid analysis of an acid hydrolysate and matrix assisted laser desorption mass spectrometry.

EXAMPLE 8

Effects of cytotoxic agent-peptide conjugates on tumor cell proliferation.

A CellTiter 96 cell proliferation kit was used according to the described
5 protocols (Promega Corporation, Madison, WI)). Culture medium (50 μ l) containing various concentrations of different peptides was added respectively in each well of the 96-well kit plates followed by 50 μ l of a suspension containing 5,000 cultured IMR-32 tumor cells. The plate was incubated at 37°C for different times up to and including 7 days in a humidified, 5% CO₂
10 atmosphere. Kit dye solution (15 μ l) was added to each well and the plate incubated the plates at 37°C for 4 hours in a humidified, 5%CO₂ atmosphere. After 4 hours, 100 μ l of the solubilization / stop solution were added to each well and incubation was continued at 37°C for 1 hour or more until complete
15 solubilization of the formazan crystals occurred. Absorbencies of individual well solutions were then measured at 570 nm wavelength using a 96 well plate reader.

EXAMPLE 9

In vitro effects of somatostatin analogs on rat pituitary GH release.

20

This is a primary system for evaluating SRIF analog potency and can be considered a somatostatin subtype 2 rat receptor related system which historically also correlates well with human subtype 2 binding.

Anterior pituitaries from adult male rats weighing 200-250g and housed
25 under controlled conditions (lights on from 0500-1900h), are dispersed using aseptic techniques by a trypsin/DNase method. The dispersed cells are diluted with sterile-filtered Dulbecco's modified Eagle medium (MEM) (Gibco Laboratories, Grand Island, NY (GIBCO)), which is supplemented with 2.5% fetal calf serum (GIBCO), 3% horse serum (GIBCO), 10% fresh rat serum
30 (stored on ice for no longer than 1h) from the pituitary donors, 1% MEM

nonessential amino acids (GIBCO), gentamycin (10 ng/ml; Sigma), and nystatin (10,000 U/ml; GIBCO). The cells are counted with a hemacytometer (approximately 2,000,000 cells per pituitary) and randomly plated at a density of 200,000 cells per well (Co-star cluster 24; Rochester Scientific Co., 5 Rochester, NY). The plated cells are maintained in the above Dulbecco's medium in a humidified atmosphere of 95% air and 5% CO₂ at 37° C for 96h.

In preparation for a hormone challenge, the cells are washed 3x with medium 199 (GIBCO) to remove old medium and floating cells. Each dose of secretagogue (diluted in siliconized test tubes) was tested in quadruplicate 10 wells in a total volume of 1 ml medium 199 containing 1% BSA (fraction V; Sigma Chemical Co., St. Louis, MO). Cells are pulsed with SS or SS analogs doses in the presence of GH-stimulatory 1nM GRH(1-29)NH₂. After 3h at 37°C in an air/carbon dioxide atmosphere (95/5%), the medium is removed and stored at 20°C until assayed for hormone content. GH in plasma and media is 15 measured by a standard double antibody RIA using components supplied by the NIDDK and the National Hormone and Pituitary Program.

EXAMPLE 10

Preparation of O-benzyl-D-Ser-O-benzyl-D-Ser-O-benzyl-D-Ser O-benzyl-D- 20 Ser-O-benzyl-D-Ser-L-Nle-O-2,6-dichlorobenzyl-D-Tyr-O-benzyl-D-Ser-S-4-methylbenzyl-L-Cys-L-Phe-D-Trp-N^ε-(2-chlorobenzylloxycarbonyl)-L-Lys-O-benzyl-L-Thr-S-4-methylbenzyl-L-Cys-O-benzyl-L-Thr-MBHA resin.

Methylbenzhydrylamine (MBHA) polystyrene resin (Bachem, Inc., 25 Torrance, CA) (0.25 mmole) was added to the reaction vessel of a CS136 automatic peptide synthesizer (CS Bio, Inc., San Carlos, CA) and swollen in methylene chloride (DCM) for approximately 1 hour. The resin was filtered and an excess of 10% diisopropylethylamine (DIPEA) was added and mixed for two minutes. The resin was filtered and again an excess amount of 10% 30 DIPEA added and mixed for 5 minutes to ensure complete neutralization of the

resin. After neutralization, the resin was washed 4 times with DCM and then the first protected amino acid, Boc-Thr(Bzl)-OH (0.75 mmol), diisopropylcarbodiimide (DIC) (0.75 mmol), and N-hydroxybenzotriazole monohydrate (HOBr) (0.75 mmol) were all dissolved in DMF and added to the 5 resin which was mixed (1 h) followed by washing 4 times with DCM.

The tBoc group was removed by adding an excess of 40% Trifluoroacetic Acid (TFA) DCM and mixed for 2 minutes. The resin was filtered and again an excess amount of 40% TFA added and mixed for 20 minutes to ensure complete removal of the N-terminal tBoc. The resin was 10 filtered and an excess of 10% diisopropylethylamine (DIPEA) was added and mixed for two minutes. The resin was filtered and again an excess amount of 10% DIPEA added and mixed for 5 minutes to ensure complete neutralization of the resin. After neutralization, the resin was washed 4 times with DCM and then following the same general coupling procedures, the following amino 15 acids were successively reacted with the growing peptide chain: Boc-S-4-methylbenzyl-L-cysteine, Boc-O-benzyl-L-threonine, N^a-Boc-N^e-(2-chlorobenzyloxycarbonyl)-L-lysine, Boc-D-tryptophan, Boc-L-phenylalanine, Boc-S-4-methylbenzyl-L-cysteine, Boc-O-benzyl-D-serine, Boc-O-2,6-dichlorobenzyl-D-tyrosine, Boc-norleucine, Boc-O-benzyl-D-serine, Boc-O-20 benzyl-D-serine, Boc-O-benzyl-D-serine, Boc-O-benzyl-D-serine, Boc-O-benzyl-D-serine. The resin was finally washed 4 times with methanol and left overnight to dry.

EXAMPLE 11

25 Preparation of D-Ser-D-Ser-D-Ser-D-Ser-D-Ser-L-Norleucine-D-Tyr-D-Ser-cyclo[L-Cys-L-Phe-D-Trp-L-Lys-L-Thr-L-Cys]-L-Thr-NH₂.

The peptidyl resin from Example 10 along with 5 mL of anisole was placed in a Teflon apparatus appropriate for anhydrous hydrogen fluoride 30 manipulation. The reaction vessel was cooled in an alcohol/dry ice bath for 5

minutes and then 35mL of gaseous anhydrous fluoride (HF) was condensed into the reaction vessel. The bath was changed from dry ice to regular ice and allowed to mix for one hour, then the HF was purged from the reaction vessel with a stream of nitrogen. The peptide was precipitated 3 times with excess 5 ethyl ether and filtered. The filtered, crude peptide was dissolved in 60-90 percent acetic acid (250 ml). A concentrated solution of iodine in methanol was added dropwise with vigorous stirring until a permanent brown coloration was formed whereupon excess iodine was removed by addition of a small quantity of ascorbic acid.

10 The solution was reduced to a volume of around 10 ml *in vacuo* and the crude peptide purified by preparative reverse phase high pressure liquid chromatography (RP-hplc) on a column (21.4 x 250 mm) of C-18 bonded silica (Dynamax 300, 8 μ m). A linear gradient elution system at a flow rate of 20 mL/min was employed: buffer A consisted of 0.1% TFA and buffer B, 0.1% 15 TFA in 80% MeCN; 20% B to 50% B was increased at 1% per minute. The separations were monitored at 280nm. The fractions containing the pure product as evidenced by analytical HPLC were pooled, concentrated *in vacuo* and subjected to lyophilization. The peptide was obtained as a fluffy white 20 powder of constant weight by lyophilization from aqueous acetic acid. Correct composition was demonstrated by amino acid analysis of an acid hydrolysate and matrix assisted laser desorption mass spectrometry.

EXAMPLE 12

Preparation of D-Ser-D-Ser-D-Ser-D-Ser-D-Ser-L-Lys-3-¹²⁵I-D-Tyr-D-Ser – 25 cyclo[L-Cys- L-Phe-D-Trp-L-Lys-L-Thr-L-Cys]-L-Thr-NH₂.

A modified Chloramine-T reaction was used to iodinate the peptide prepared in Example 11. Phosphate buffer (200 μ L of 0.05M) was added to a septum-sealed vial containing 20 mCi of ¹²⁵Iodine and this served as the 30 reaction vessel. Once neutralized, 9.411×10^{-8} moles of peptide dissolved in

100 μ L phosphate buffer were injected into the reaction vial. To start the iodination reaction, 4.29×10^{-7} moles of chloramine-T were injected into the reaction vial in 50 μ L of buffer. The reaction vessel was vortexed rapidly for 15-20 seconds and then the reaction halted by injecting 4.3×10^{-6} moles of 5 sodium metabisulfite in 50 μ L.

Purification:

A C18 SepPak Lite cartridge (Waters Corp., Milford, Mass.) was used to purify the peptide from reactants. The SepPak was activated by washing with 10 mL of absolute ethanol at a flow rate of 1 mL / min. The SepPak was 10 then washed with 10 mL of H₂O. The radioactive reaction mixture was then applied to the SepPak followed by 3 x 3-mL washes of the reaction vessel with 5% ethanol. The peptide was separated from the rest of the reactants by 15 washing the SepPak with 10 mL of 20% ethanol. The peptide was finally eluted off the column in 250 μ L aliquots using a solution of 80% ethanol in 0.01 N HCL and collected into 2 mL sterile screw cap vials.

EXAMPLE 13

Preparation of O-benzyl-D-Ser-O-benzyl-D-Ser-O-benzyl-D-Ser-O-benzyl-D-
Ser-O-benzyl-D-Ser-O-benzyl-D-Ser-O-benzyl-D-Ser-O-benzyl-D-Ser-O-
20 benzyl-D-Ser-O-benzyl-D-Ser-N^ε-(2-chlorobenzylloxycarbonyl)-L-Lys-O-2,6-
dichlorobenzyl-L-Tyr-O-2,6-dichlorobenzyl-L-Tyr-S-4-methylbenzyl-L-Cys-L-
Phe-D-Trp-N^ε-(2-chlorobenzylloxycarbonyl)-L-Lys-O-benzyl-L-Thr-S-4-
methylbenzyl-L-Cys-O-benzyl-L-Thr-MBHA resin.

25 Methylbenzhydrylamine (MBHA) polystyrene resin (Bachem, Inc.,
Torrance, CA) (0.25 mmole) was added to the reaction vessel of a CS136
automatic peptide synthesizer (CS Bio, Inc., San Carlos, CA) and swollen in
methylene chloride (DCM) for approximately 1 hour. The resin was filtered
and an excess of 10% diisopropylethylamine (DIPEA) was added and mixed
30 for two minutes. The resin was filtered and again an excess amount of 10%

DIEA added and mixed for 5 minutes to ensure complete neutralization of the resin. After neutralization, the resin was washed 4 times with DCM and then the first protected amino acid, Boc-Thr(Bzl)-OH (0.75 mmol), diisopropylcarbodiimide (DIC) (0.75 mmol), and N-hydroxybenzotriazole monohydrate (HOBr) (0.75 mmol) were all dissolved in DMF and added to the resin which was mixed (1 h) followed by washing 4 times with DCM.

The tBoc group was removed by adding an excess of 40% trifluoroacetic acid (TFA) DCM and mixed for 2 minutes. The resin was filtered and again an excess amount of 40% TFA added and mixed for 20 minutes to ensure complete removal of the N-terminal tBoc. The resin was filtered and an excess of 10% diisopropylethylamine (DIEA) was added and mixed for two minutes. The resin was filtered and again an excess amount of 10% DIEA added and mixed for 5 minutes to ensure complete neutralization of the resin. After neutralization, the resin was washed 4 times with DCM and then following the same general coupling procedures, the following amino acids were successively reacted with the growing peptide chain Boc-S-4-methylbenzyl-L-cysteine, Boc-O-benzyl-L-threonine, N^a-Boc-N^e-(2-chlorobenzylloxycarbonyl)-L-lysine, Boc-D-tryptophan, Boc-L-phenylalanine, Boc-S-4-methylbenzyl-L-cysteine, Boc-O-2,6-dichlorobenzyl-D-tyrosine, Boc-O-2,6-dichlorobenzyl-D-tyrosine, N^a-Boc-N^e-(2-chlorobenzylloxycarbonyl)-L-lysine, Boc-O-benzyl-D-serine, Boc-O-benzyl-D-serine, Boc-O-benzyl-D-serine, Boc-O-benzyl-D-serine, Boc-O-benzyl-D-serine, Boc-O-benzyl-D-serine, Boc-O-benzyl-D-serine, Boc-O-benzyl-D-serine. The final Boc group was removed, the peptide neutralized, and washed using the same general procedure as above.

EXAMPLE 14

Preparation of DOTA-O-benzyl-D-Ser-O-benzyl-D-Ser-O-benzyl-D-Ser-O-benzyl-D-Ser -O-benzyl-D-Ser-O-benzyl-D-Ser-O-benzyl-D-Ser-O-benzyl-D-Ser-O-benzyl-D-Ser-O-benzyl-D-Ser-N^ε-(2-chlorobenzylloxycarbonyl)-L -Lys-
5 O-2,6-dichlorobenzyl-L-Tyr-O-2,6-dichlorobenzyl-L-Tyr -S-4-methylbenzyl-L-Cys-L-Phe- D-Trp-N^ε-(2-chlorobenzylloxycarbonyl)-L-Lys- O-benzyl-L-Thr-S-4-methylbenzyl-L-Cys-O-benzyl-L-Thr-MBHA resin.

10 In order to couple DOTA, which is very insoluble in organic solvents including DMF and DMSO alone, to the free amino group of the growing chain, a novel procedure utilizing the unexpected solubilizing effects of HOBT was used. First, 5 mmol of HOBT was dissolved in a beaker containing 50-75 mL of DMSO, then 1.25 mmol of DOTA were added and the suspension
15 vigorously mixed until the DOTA went into solution. DIC (1.25 mmol) was added to the beaker and this mixture was added to the peptidyl resin , mixed overnight, and then washed 4 times with DMF and 4 times with methanol.

EXAMPLE 15

20 Preparation of DOTA-D-Ser-D-Ser-D-Ser-D-Ser-D-Ser-D-Ser-D-Ser-D-Ser-D-Ser-D-Ser-L-Lys-D-Tyr-D-Tyr-cyclo[L-Cys-L-Phe-D-Trp-L-Lys-L-Thr-L-Cys]-L-Thr-NH₂.

25 The protected peptide resin described in Example 14 was subjected to cleavage from the resin and removal of side-chain protecting groups using liquid HF as described in Example 12. The free peptide was then cyclized and purified also as described in Example 12.

EXAMPLE 16

Preparation of D-O-benzyl-Ser-O-benzyl-Ser-D-O-benzyl-Ser-O-benzyl-Ser-D-O-benzyl-Ser-O-benzyl-Ser-D-O-benzyl-Ser-O-benzyl-Ser-D-O-benzyl-Ser-O-benzyl-Ser-D-O-
5 dichlorobenzyl-Tyr-D-O-benzyl-Ser-Gln-Trp-Ala-Val- β -Ala-tosyl-His-Phe-Nle-MBHA resin.

Methylbenzhydrylamine (MBHA) polystyrene resin (Bachem, Inc.,
Torrance, CA) (0.25 mmole) was added to the reaction vessel of a CS136
10 automatic peptide synthesizer (CS Bio, Inc., San Carlos, CA) and the peptide
was assembled by successive additions of amino acid derivatives as described
in Example 13.

EXAMPLE 17

15 D-Ser-Ser-D-Ser-Ser-D-Ser-Ser-D-Tyr-D-Ser-Gln-Trp-Ala-Val- β -
Ala-His-Phe-Nle-NH₂.

The protected peptide resin described in Example 16 was subjected to
cleavage from the resin and removal of side-chain protecting groups using
20 liquid HF as described in Example 12. The free peptide was then purified also
as described in Example 12.

EXAMPLE 18

In vitro effects of bombesin agonist analogs on guinea pig pancreatic acinar
25 cell amylase release.

Dispersed acini from one guinea pig pancreas were suspended in 150 ml
of standard incubation solution and samples (250 ml) were incubated for 30
min at 37°C, and amylase release was determined by the Phadebas reagent
30 method. Amylase release was calculated as the percentage of amylase activity

in the acini at the beginning of the incubation that was released into the extracellular medium during the incubation. Various concentrations of bombesin standard and analogs were incubated at various concentrations in order to determine half-maximal stimulation values (EC₅₀).

5

EXAMPLE 19

Biodistribution of ¹²⁵I labeled JF-08-73 (Succinate-D-Asp-D-Ser-D-Ser-D-Ser-D-Ser-D-Asp-D-Ser-D-Ser-D-Ser-L-Lys-D-Tyr-D-Tyr-cyclo[L-Cys-L-Phe-D-Trp-L-Lys-L-Thr-L-Cys]-L-Thr-NH₂ and JF-08-53 (D-10 Ser-D-Ser-D-Ser-D-Ser-L-Lys-D-Tyr-D-Ser-cyclo[L-Cys-L-Phe-D-Trp-L-Lys-L-Thr-L-Cys]-L-Thr-NH₂.

The peptides were radiolabeled as described in Example 12. The second screw cap vial containing the highest amounts of radioactive peptide was 15 diluted with phosphate buffered saline to achieve a final concentration of less than 3% ethanol. The solution was divided into three equal parts not exceeding 2 mL and injected intraperitoneally into each rat. Urine and feces were collected on an adsorbent pad overnight. At 24 hours, the rats were killed, dissected and each of the below organs were weighed and counted in a dose 20 calibrator. The results are shown in Fig. 2.

EXAMPLE 20

Preparation of thiocolchicine.

25 Colchicine (1.25 mmole) was dissolved in 10.0 mL of water to which was added sodium methanethiolate (7.13 mmol). The yellow solution was stirred for 24 hours, then extracted 3x with ETOH:Chloroform (1:1), then evaporated to yield the desired compound as yellow crystals.

EXAMPLE 21

Preparation of deacetylthiocolchicine.

5 To the product from Example 1, 5.0 mL of methanol and 5.0 mL of 2N HCl were added and the solution refluxed 18 hours under dry nitrogen. The methanol was distilled off, the residual solution was reneutralized with NaOH, extracted 3 times with chloroform, evaporated, and finally lyophilized from acetonitrile/water as a yellow powder.

10

EXAMPLE 22

Preparation of deacetylthiocolchicine-3-thiodipropionyl-N^ε-(2-chlorobenzylloxycarbonyl)-L-Lys-O-benzyl-D-Ser-O-benzyl-D-Ser-O-benzyl-D-Ser-O-benzyl-D-Ser-O-benzyl-D-Ser-O-benzyl-D-Ser-O-benzyl-D-Ser-O-benzyl-D-Ser-O-benzyl-D-Ser-O-benzyl-D-Ser-L-Nle-O-2,6-dichlorobenzyl-D-Tyr-O-benzyl-D-Ser-S-4-methylbenzyl-L-Cys-L-Phe-D-Trp-N^ε-(2-chlorobenzylloxycarbonyl)-L-Lys-O-benzyl-L-Thr-S-4-methylbenzyl-L-Cys-O-benzyl-L-Thr-MBHA resin.

20

Methylenhydrylamine (MBHA) polystyrene resin (Bachem, Inc., Torrance, CA) (0.25 mmole) was added to the reaction vessel of a CS136 automatic peptide synthesizer (CS Bio, Inc., San Carlos, CA) and swollen in methylene chloride (DCM) for approximately 1 hour. The resin was filtered and an excess of 10% diisopropylethylamine (DIPEA) was added and mixed for two minutes. The resin was filtered and again an excess amount of 10% DIPEA added and mixed for five minutes to ensure complete neutralization of the resin. After neutralization, the resin was washed 4 times with DCM and then the first protected amino acid, Boc-Thr(Bzl)-OH (0.75 mmol),

diisopropylcarbodiimide (DIC) (0.75 mmol), and N-hydroxybenzotriazole monohydrate (HOBr) (0.75 mmol) were all dissolved in DMF and added to the resin which was mixed (1 h) followed by washing 4 times with DCM.

The tBoc group was removed by adding an excess of 40% TFA in dichloromethane.

5 Trifluoroacetic Acid (TFA) DCM and mixed for 2 minutes. The resin was
filtered and again an excess amount of 40% TFA added and mixed for 20
minutes to ensure complete removal of the N-terminal tBoc. The resin was
filtered and an excess of 10% diisopropylethylamine (DIPEA) was added and
mixed for two minutes. The resin was filtered and again an excess amount of
10 10% DIPEA added and mixed for 5 minutes to ensure complete neutralization
of the resin. After neutralization, the resin was washed 4 times with DCM and
then following the same general coupling procedures, the following amino
acids were successively reacted with the growing peptide chain: Boc-S-4-
15 methylbenzyl-L-cysteine, Boc-O-benzyl-L-threonine, N^a-Boc-N^e-(2-
chlorobenzylloxycarbonyl)-L-lysine, Boc-D-tryptophan, Boc-L-phenylalanine,
Boc-S-4-methylbenzyl-L-cysteine, Boc-O-benzyl-D-serine, Boc-O-2,6-
dichlorobenzyl-D-tyrosine, Boc-norleucine, Boc-O-benzyl-D-serine, Boc-O-
benzyl-D-serine, Boc-O-benzyl-D-serine, Boc-O-benzyl-D-serine, Boc-O-
20 benzyl-D-serine, Boc-O-benzyl-D-serine, Boc-O-benzyl-D-serine, N^a-Boc-N^e-
(2-chlorobenzylloxycarbonyl)-L-lysine, 3-thiodipropionic acid, and finally
without the tBoc deprotection step, deacetylthiocolchicine was coupled in
DCM/Dic. The resin was finally washed 4 times with methanol and left
overnight to dry.

EXAMPLE 23

Preparation of Deacetylthiocolchicine-3-thiodipropionyl-D-Ser-D-Ser-D-Ser-D-Ser-D-Ser-D-Ser-D-Ser-D-Ser-L-Norleucine-D-Tyr-D-Ser-cyclo[L-Cys-L-Phe-D-Trp-L-Lys-L-Thr-L-Cys]-L-Thr-NH₂.

5

The peptidyl resin from Example 10 along with 5 mL of anisole was placed in a Teflon apparatus appropriate for anhydrous hydrogen fluoride manipulation. The reaction vessel was cooled in an alcohol/dry ice bath for 5 minutes and then 35 mL of gaseous anhydrous fluoride (HF) was condensed into the reaction vessel. The bath was changed from dry ice to regular ice and allowed to mix for one hour, then the HF was purged from the reaction vessel with a stream of nitrogen. The peptide was precipitated 3 times with excess ethyl ether and filtered. The filtered, crude peptide was dissolved in 60-90 percent acetic acid (250 ml). A concentrated solution of iodine in methanol was added dropwise with vigorous stirring until a permanent brown coloration was formed whereupon excess iodine was removed by addition of a small quantity of ascorbic acid.

The solution was reduced to a volume of around 10 ml *in vacuo* and the crude peptide purified by preparative reverse phase high pressure liquid chromatography (RP-hplc) on a column (21.4x250 mm) of C-18 bonded silica (Dynamax 300, 8 μ m). A linear gradient elution system at a flow rate of 20 mL/min was employed: buffer A consisted of 0.1% TFA and a buffer B, 0.1% TFA in 80% MeCN; 20% B to 50% B was increased at 1% per minute. The separations were monitored at 280nm. The fractions containing the pure product as evidenced by analytical HPLC were pooled, concentrated *in vacuo* and subjected to lyophilization. The peptide was obtained as a fluffy yellow powder of constant weight by lyophilization from aqueous acetic acid. Correct composition was demonstrated by amino acid analysis of an acid hydrolysate and matrix assisted laser desorption mass spectrometry.

25
30

EXAMPLE 24

Preparation of the acyl chloride of camptothecin.

Camptothecin (0.574 mmole) was suspended in 30 mL anhydrous DCM
5 in a 100 mL RB flask. To this slurry, DMAP (2,455 mmole) dissolved in 20
mL anhydrous DCM was added over 20 minutes at 0°C under a dry nitrogen
atmosphere. Phosgene (0.965 mmole) was added to the slurry and mixed for
30 minutes at 0°C and 2 hours at RT. Excess phosgene and methylene chloride
were evaporated and the acyl chloride of camptothecin dissolved in methylene
10 chloride.

EXAMPLE 25

Preparation of Camptothecin-carbonyl-Sar-D-tert-butyl-Ser-Norleucine-D-tert-
butyl-Tyr-D-tert-butyl-Ser-S-trityl-Cys-Phe-D-Trp-epsilon-tert-
15 butyloxycarbonyl-Lys-tert-butyl-Thr-S-trityl-Cys-tert-butyl-Thr-Rink-amide-
resin.

Rink amide MBHA polystyrene resin (0.063 mmole) [4-(2', 4'-
dimethoxyphenyl-Fmoc-(aminomethyl)phenoxyacetamido-norleucyl-
20 methylbenzhydrylamine resin, 100-200 mesh, Novabiochem, San Diego, C]
was added to the reaction vessel of a CS136 automatic peptide synthesizer (CS
Bio, Inc., San Carlos, CA) and swollen in DMF for approximately 1 hour. The
resin was filtered and an excess of 20% piperidine in DMF was added and
mixed for 2 minutes. The resin was filtered and again an excess amount of
25 20% piperidine added and mixed for 20 minutes to ensure complete removal of
the resin Fmoc group. After deprotection, the resin was washed 4 times with
DMF and then the first protected amino acid, Fmoc-Thr(tBut) (0.188 mmol),
diisopropylcarbodiimide (DIC) (0.188 mmol), and N-hydroxybenzotriazole
monohydrate (HOBt) (0.188 mmol) were all dissolved in DMF and added to
30 the resin which was mixed (1 h) followed by washing 4 times with DMF.

The Fmoc group was again removed by treatment with 20% piperidine/DMF solution and, following the same general coupling procedures, the following amino acids were successively reacted with the growing peptide chain: Fmoc-S-trityl-L-cysteine, Fmoc-O-t-butyl-L-threonine-N^a-Fmoc-N^c-

5 Boc-L-lysine, N^a-Fmoc-Nⁱⁿ-Boc-D-tryptophan, Fmoc-L-phenylalanine, Fmoc-S-trityl-L-cysteine, Fmoc-O-t-butyl-D-serine, Fmoc-O-t-butyl-D-tyrosine, N^a-Fmoc-norleucine, Fmoc-O-t-butyl-D-serine, Fmoc-sarcosine. After removal of the final Fmoc group, Camptothecin acyl chloride from EXAMPLE 24 was

10 added to the resin and mixed overnight, washed with copious amounts of DMF followed by DCM then methanol. After a final filtration the derivatized resin air dried overnight.

EXAMPLE 26

Preparation of camptothecin-carbonyl-Sar-D-Ser-Nle-D-Tyr-D-Ser-cyclo[Cys-
15 Phe-D-Trp-Lys-Thr-Cys]-Thr-amide.

The camptothecin-peptide resin prepared in Example 25 (0.063 mmol) was placed in a round bottomed flask to which was added 15 ml of a solution of trifluoroacetic acid (TFA) containing water (2.5%), 1,2-ethanedithiol (2.5%), and triisopropylsilane (1%). The suspension was agitated (2 h), filtered, and washed several times with TFA. The TFA was evaporated *in vacuo* and ether added to the resulting oil to give a yellow powder which was then dissolved in 60% acetic acid (250 ml). A concentrated solution of iodine in methanol was added dropwise with vigorous stirring until a permanent
20 brown coloration was formed whereupon excess iodine was removed by addition of a small quantity of ascorbic acid.

25

The solution was reduced to a volume of around 10 ml *in vacuo* and the crude camptothecin peptide purified by preparative reverse phase high pressure liquid chromatography (RP-hplc) on a column (21.4 x 250 mm) of C-18 bonded silica (Dynamax 300, 8 μ m). A linear gradient elution system at a flow
30

rate of 20 mL/min was employed: buffer A consisted of 0.1% TFA and buffer B, 0.1% TFA in 80% MeCN; 20% B to 50% B was increased at 1% per min. The separation were monitored at 280nm. The fractions containing the pure product as evidenced by analytical hplc were pooled, concentrated *in vacuo*, 5 and subjected to lyphilization. The peptide was obtained as a fluffy white powder of constant weight by lyphilization from aqueous acetic acid. Correct composition was demonstrated by amino acid analysis of an acid hydrolysate and matrix assisted laser desorption mass spectrometry.

Table 1. Ability of various somatostatin analog conjugates to inhibit GH release from primary cultures of rat pituitary cells. For comparison, natural somatostatin -14 has an IC₅₀ value of 0.15 nM (ND = not done).

Peptide Synthesis Code	IC ₅₀ (nM)
Somatostatin-14	0.15
JF-07-100	0.62
JF-08-87A	0.16
JF-09-35	ND
D-Ser-D-Ser-D-Ser-D-Ser-D-Ser-L-Lys-D-Tyr-D-Tyr-cyclo[L-Cys-L-Phe-D-Trp-L-Lys-L-Thr-L-Cys]-L-Thr-NH ₂	0.14
D-Ser-D-Ser-D-Ser-D-Ser-D-Ser-D-Ser-D-Ser-D-Ser-D-Ser-L-Lys-D-Tyr-D-Tyr-cyclo[L-Cys-L-Phe-D-Trp-L-Lys-L-Thr-L-Cys]-L-Thr-NH ₂	0.12
Acetyl-D-Ser-D-Ser-D-Ser-D-Ser-D-Ser-D-Ser-D-Ser-D-Ser-D-Ser-L-Lys-D-Tyr-D-Tyr-cyclo[L-Cys-L-Phe-D-Trp-L-Lys-L-Thr-L-Cys]-L-Thr-NH ₂	0.57
D-Ser-D-Ser-D-Ser-D-Ser-D-Ser-L-Lys-D-Ser-D-Ser-cyclo[L-Cys-L-Phe-D-Trp-L-Lys-L-Thr-L-Cys]-L-Thr-NH ₂	3.29
DOTA-D-Ser-D-Ser-D-Ser-D-Ser-D-Ser-D-Ser-D-Ser-D-Ser-D-Ser-L-Lys-D-Tyr-D-Tyr-cyclo[L-Cys-L-Phe-D-Trp-L-Lys-L-Thr-L-Cys]-L-Thr-NH ₂	0.71

Table 1. (Cont.)

Peptide Synthesis Code	IC ₅₀ (nM)
D-Ser-D-Ser-D-Ser-D-Ser-D-Ser-D-Ser-D-Ser-D-Ser-D-Ser-D-Ser-D-Ser-D-Ser-D-Ser-L-Lys-D-Tyr-D-Tyr-cyclo[L-Cys-L-Phe-D-Trp-L-Lys-L-Thr-L-Cys]-L-Thr-NH ₂	0.32
D-Ser-D-Ser-D-Ser-D-Ser-D-Ser-D-Ser-D-Ser-D-Ser-D-Ser-D-Ser-D-Ser-D-Ser-D-Ser-D-Ser-D-Ser-L-Lys-D-Tyr-D-Tyr-cyclo[L-Cys-L-Phe-D-Trp-L-Lys-L-Thr-L-Cys]-L-Thr-NH ₂	ND
D-Ser-D-Ser-D-Ser-D-Ser-D-Ser-L-Lys-D-Tyr-D-Ser-cyclo[L-Cys-L-Phe-D-Trp-L-Lys-L-Thr-L-Cys]-L-Thr-NH ₂	0.086
D-Gln-D-Gln-D-Gln-D-Gln-D-Gln-D-Gln-D-Gln-D-Gln-D-Gln-L-Lys-D-Tyr-D-Tyr-cyclo[L-Cys-L-Phe-D-Trp-L-Lys-L-Thr-L-Cys]-L-Thr-NH ₂	0.24
3-N,N-Dimethylaminobenzoic-D-Ser-D-Ser-D-Ser-D-Ser-D-Ser-D-Ser-D-Ser-D-Ser-D-Ser-D-Ser-D-Ser-D-Ser-D-Ser-D-Ser-L-Lys-D-Tyr-D-Tyr-cyclo[L-Cys-L-Phe-D-Trp-L-Lys-L-Thr-L-Cys]-L-Thr-NH ₂	0.27
Succinate-D-Asp-D-Ser-D-Ser-D-Ser-D-Ser-D-Asp-D-Ser-D-Ser-D-Ser-D-Ser-L-Lys-D-Tyr-D-Tyr-cyclo[L-Cys-L-Phe-D-Trp-L-Lys-L-Thr-L-Cys]-L-Thr-NH ₂	0.51
D-Ser-D-Ser-D-Ser-D-Ser-D-Ser-L-Norleucine-D-Tyr-D-Ser-cyclo[L-Cys-L-Phe-D-Trp-L-Lys-L-Thr-L-Cys]-L-Thr-NH ₂	0.094
D-Ser-D-Ser-D-Ser-D-Ser-D-Ser-L-4Pal-D-Tyr-D-Ser-cyclo[L-Cys-L-Phe-D-Trp-L-Lys-L-Thr-L-Cys]-L-Thr-NH ₂	0.18
D-Ser-D-Ser-D-Ser-D-Ser-D-Ser-L-Gln-D-Tyr-D-Ser-cyclo[L-Cys-L-Phe-D-Trp-L-Lys-L-Thr-L-Cys]-L-Thr-NH ₂	0.45

Table 1. (Cont.).

Peptide Synthesis Code	IC ₅₀ (nM)
D-Ser-D-Ser-D-Ser-D-Ser-D-Ser-L-Asp-D-Tyr-D-Ser-cyclo[L-Cys-L-Phe-D-Trp-L-Lys-L-Thr-L-Cys]-L-Thr-NH ₂	7.55
Succinate-D-Asp-D-Ser-D-Ser-D-Ser-D-Ser-L-Norleucine-D-Tyr-D-Ser-cyclo[L-Cys-L-Phe-D-Trp-L-Lys-L-Thr-L-Cys]-L-Thr-NH ₂	0.52
Succinate-D-Ser-D-Ser-D-Ser-D-Ser-D-Ser-L-Ser-D-Tyr-D-Ser-cyclo[L-Cys-L-Phe-D-Trp-L-Lys-L-Thr-L-Cys]-L-Thr-NH ₂	5.6
Succinate-D-Ser-D-Ser-D-Ser-D-Ser-D-Ser-L-2-Aminobutyric acid-D-Tyr-D-Ser-cyclo[L-Cys-L-Phe-D-Trp-L-Lys-L-Thr-L-Cys]-L-Thr-NH ₂	0.61
D-Ser-D-Ser-D-Ser-D-Ser-D-Ser-L-Thr-D-Tyr-D-Ser-cyclo[L-Cys-L-Phe-D-Trp-L-Lys-L-Thr-L-Cys]-L-Thr-NH ₂	0.72
Succinate-D-Asp-D-Ser-D-Ser-D-Ser-D-Ser-L-Thr-D-Tyr-D-Ser-cyclo[L-Cys-L-Phe-D-Trp-L-Lys-L-Thr-L-Cys]-L-Thr-NH ₂	5.17
D-Ser-D-Ser-D-Ser-D-Ser-D-Ser-L-hSer-D-Tyr-D-Ser-cyclo[L-Cys-L-Phe-D-Trp-L-Lys-L-Thr-L-Cys]-L-Thr-NH ₂	0.55
Mercaptoacetate-Gly-Gly-D-Asp-D-Ser-D-Ser-D-Ser-D-Ser-L-4Pal-D-Tyr-D-Ser-cyclo[L-Cys-L-Phe-D-Trp-L-Lys-L-Thr-L-Cys]-L-Thr-NH ₂	0.97
D-Ser-D-Ser-D-Ser-D-Gln-D-Ser-D-Ser-D-Gln-D-Ser-D-Ser-D-Ser-D-Gln-L-Norvaline-D-Tyr-D-Ser-cyclo[L-Cys-L-Phe-D-Trp-L-Lys-L-Thr-L-Cys]-L-Thr-NH ₂	0.93
D-Ser-L-Ser-D-Ser-L-Ser-D-Ser-L-Ser-D-Ser-L-Ser-D-Ser-L-2-Aminobutyric acid-D-Tyr-D-Ser-cyclo[L-Cys-L-Phe-D-Trp-L-Lys-L-Thr-L-Cys]-L-Thr-NH ₂	ND

Other Embodiments:

Although the present invention has been described with reference to preferred embodiments, one skilled in the art can easily ascertain its essential characteristics and, without departing from the spirit and scope thereof, can 5 make various changes and modifications of the invention to adapt it to various usages and conditions. Those skilled in the art will recognize or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed in the scope of the present invention.

10 All publications, patents, and applications mentioned in the specification are herein incorporated by reference.

We claim:

Claims

1. A peptide agent having the formula:

X-Y-Z-Q, wherein:

X is selected from the group consisting of cytotoxic agents, therapeutic agents, 5 detectable labels, and chelating groups, or is omitted;

Y is a peptide that increases the hydrophilic biodistribution of said agent, a hydrophilic polymer that includes a linker for X, or is omitted;

Q is a peptide having biological activity; and

Z is a linking peptide that, when bonded to Q at the N-terminus or at a 10 compatible side-chain amino group of Q, preserves at least 50% of the biological activity of Q, Z having the formula:

A-B-C-E-F, wherein:

A is D-Lys, D-Tyr, D-Ser, or L-Ser, or is deleted;

B is D-Lys or D-Tyr, or is deleted;

15 C is Lys, Ser, hSer, Thr, Nle, Abu, Nva, (2, 3, or 4) 3-pyridyl-Ala (Pal), Orn, Dab, Dap, 4-NH₂-Phe, D-4-OH-Pro, or L-4-OH-Pro, or is deleted;

E is D-Lys, D-Tyr, D-Ser, D-4-OH-Pro, L-4-OH-Pro, 3-iodo-D-Tyr, 3-5 diiodo-D-Tyr, 3-astatine-D-Tyr, 3-5 astatine-D-Tyr, 3-bromo-D-Tyr, 3-5 dibromo-D-Tyr, D-Asn, L-Asn, D-Asp, L-Asp, D-Glu, L-Glu, D-Gln, or L-Gln; and 20

F is D-Lys, D-Tyr, D-Ser, L-Ser, D-4-OH-Pro, L-4-OH-Pro, 3-iodo-D-Tyr, 3-5 diiodo-D-Tyr, 3-astatine-D-Tyr, 3-5 astatine-D-Tyr, 3-bromo-D-Tyr, 3-5 dibromo-D-Tyr, D-Asn, L-Asn, D-Asp, L-Asp, D-Glu, L-Glu, D-Gln, or L-Gln;

provided that when A, B, C, and E are Tyr, Tyr, Lys, and Tyr, respectively, F is not Lys; and when A, B, C, and E are Lys, Tyr, Lys, and Tyr, respectively, E is not Tyr or Lys; and when A and B are deleted, and C and E are Lys and Tyr, respectively, F is not Tyr or Lys.

5 2. A peptide agent having the formula:

X-Y-Z-Q, wherein:

X is a cytotoxic agent or a therapeutic agent;

Y is a peptide that increases the hydrophilic biodistribution of said agent, a hydrophilic polymer that includes a linker for X, or is omitted;

10 Q is a peptide having biological activity; and

Z is a linking peptide that, when bonded to Q at the N-terminus or at a compatible side-chain amino group of Q, preserves at least 50% of the biological activity of Q, Z having the formula:

A-B-C-E-F, wherein:

15 A is D-Lys, D-Tyr, D-Ser, or L-Ser, or is deleted;

B is D-Lys or D-Tyr, or is deleted;

C is Lys, Ser, hSer, Thr, Nle, Abu, Nva, (2, 3, or 4) 3-pyridyl-Ala (Pal), Orn, Dab, Dap, 4-NH₂-Phe, D-4-OH-Pro, or L-4-OH-Pro, or is deleted;

20 E is D-Lys, D-Tyr, D-Ser, D-4-OH-Pro, L-4-OH-Pro, 3-iodo-D-Tyr, 3-5 diiodo-D-Tyr, 3-astatine-D-Tyr, 3-5 astatine-D-Tyr, 3-bromo-D-Tyr, 3-5 dibromo-D-Tyr, D-Asn, L-Asn, D-Asp, L-Asp, D-Glu, L-Glu, D-Gln, or L-Gln; and

F is D-Lys, D-Tyr, D-Ser, L-Ser, D-4-OH-Pro, L-4-OH-Pro, 3-iodo-D-Tyr, 3-5 diiodo-D-Tyr, 3-astatine-D-Tyr, 3-5 astatine-D-Tyr, 3-bromo-D-Tyr, 3-5 dibromo-D-Tyr, D-Asn, L-Asn, D-Asp, L-Asp, D-Glu, L-Glu, D-Gln, or L-Gln;

5 provided that when A, B, C, and E are Tyr, Tyr, Lys, and Tyr, respectively, F is not Lys; and when A, B, C, and E are Lys, Tyr, Lys, and Tyr, respectively, E is not Tyr or Lys; and when A and B are deleted, and C and E are Lys and Tyr, respectively, F is not Tyr or Lys.

10 3. The peptide agent of claim 2, wherein Y is a peptide that increases the hydrophilic biodistribution of said agent.

4. The peptide agent of claim 3, wherein Y is of the formula:

U(V-V)_n, wherein:

U is D-Pro, L-Pro, D-4-OH-Pro, L-4-OH-Pro, Sar, or (NH₂-(CH₂)_m-COOH) where m=2-10, inclusive, or is deleted;

15 each V is independently selected from the group consisting of: D-Ser, L-Ser, D-Thr, L-Thr, D-Gln, L-Gln, D-Asn, L-Asn, D-4-OH-Pro, and L-4-OH-Pro; and

n is an integer from 1 to 50, inclusive.

20 5. The peptide agent of claim 4, wherein each V is independently D-Ser or L-Ser.

6. The peptide agent of claim 2, wherein Y is a hydrophilic polymer.

25 7. The peptide agent of claim 6, wherein Y is polyethylene glycol, polyvinyl acetate, polyvinyl alcohol, HPMA (N-(2-hydroxypropyl) methacrylamide) or HPMA copolymers, α , β -poly(N-hydroxyethyl)-DL-aspartamide (PHEA), or α , β -poly(N-hydroxypropyl)-DL-aspartamide.

8. The peptide agent of claim 2, wherein X is a cytotoxic agent.
9. The peptide agent of claim 8, wherein X is an antimetabolic agent.
10. The peptide agent of claim 8, wherein X is a methotrexate.
11. The peptide agent of claim 8, wherein X is selected from the group consisting of the following compounds and their derivatives: doxorubicin, methotrexate, camptothecin, homocamptothecins, rhizoxins, dolistatins, paclitaxol, and maytansinoids.
12. The peptide agent of claim 2, wherein Q is a somatostatin peptide.
13. The peptide agent of claim 2, wherein Q is a bombesin peptide.
- 10 14. The peptide agent of claim 2, wherein Z is D-Ser-Lys-D-Tyr-D-Tyr.
15. The peptide agent of claim 2, wherein Z is D-Ser-Lys-D-Tyr-D-Ser.
16. The peptide agent of claim 2, wherein Z is D-Ser-Ser-D-Lys-D-Ser.
17. The peptide agent of claim 2, wherein Z is D-Ser-Ser-D-Lys-Ser.
18. The peptide agent of claim 2, wherein Z is D-Ser-Nle-D-Tyr-D-Ser.
- 15 19. The peptide agent of claim 2, wherein Z is D-Ser-Pal-D-Tyr-D-Ser.
20. The peptide agent of claim 2, wherein Z is D-Ser-Thr-D-Tyr-D-Ser.
21. A method of treating a disease comprising administering to a subject suffering from said disease a therapeutically effective amount of a peptide agent having the formula:

20 X-Y-Z-Q, wherein:

X is a cytotoxic agent or a therapeutic agent;

Y is a peptide that increases the hydrophilic biodistribution of said agent, a hydrophilic polymer that includes a linker for X, or is omitted;

Q is a peptide having biological activity; and

Z is a linking peptide that, when bonded to Q at the N-terminus or at a compatible side-chain amino group of Q, preserves at least 50% of the biological activity of Q, Z having the formula:

5 A-B-C-E-F, wherein:

A is D-Lys, D-Tyr, D-Ser, or L-Ser, or is deleted;

B is D-Lys or D-Tyr, or is deleted;

C is Lys, Ser, hSer, Thr, Nle, Abu, Nva, (2, 3, or 4) 3-pyridyl-Ala (Pal), Orn, Dab, Dap, 4-NH₂-Phe, D-4-OH-Pro, or L-4-OH-Pro, or is deleted;

10 E is D-Lys, D-Tyr, D-Ser, D-4-OH-Pro, L-4-OH-Pro, 3-iodo-D-Tyr, 3-5 diiodo-D-Tyr, 3-astatine-D-Tyr, 3-5 astatine-D-Tyr, 3-bromo-D-Tyr, 3-5 dibromo-D-Tyr, D-Asn, L-Asn, D-Asp, L-Asp, D-Glu, L-Glu, D-Gln, or L-Gln; and

15 F is D-Lys, D-Tyr, D-Ser, L-Ser, D-4-OH-Pro, L-4-OH-Pro, 3-iodo-D-Tyr, 3-5 diiodo-D-Tyr, 3-astatine-D-Tyr, 3-5 astatine-D-Tyr, 3-bromo-D-Tyr, 3-5 dibromo-D-Tyr, D-Asn, L-Asn, D-Asp, L-Asp, D-Glu, L-Glu, D-Gln, or L-Gln;

provided that when A, B, C, and E are Tyr, Tyr, Lys, and Tyr, respectively, F is not Lys; and when A, B, C, and E are Lys, Tyr, Lys, and Tyr, respectively, E is not Tyr or Lys; and when A and B are deleted, and C and E are Lys and Tyr, respectively, F is not Tyr or Lys.

22. The method of claim 21, wherein Y is a peptide that increases the hydrophilic biodistribution of said agent .

23. The method of claim 21, wherein Y is of the formula:

U(V-V)_n, wherein:

U is D-Pro, L-Pro, D-4-OH-Pro, L-4-OH-Pro, Sar, or (NH₂-(CH₂)_m-COOH) where m=2-10, inclusive, or is deleted;

5 each V is independently selected from the group consisting of: D-Ser, L-Ser, D-Thr, L-Thr, D-Gln, L-Gln, D-Asn, L-Asn, D-4-OH-Pro, and L-4-OH-Pro; and

n is an integer from 1 to 50, inclusive.

24. The method of claim 23, wherein each V is independently D-Ser or
10 L-Ser.

25. The method of claim 21, wherein Y is a hydrophilic polymer.

26. The method of claim 25, wherein Y is polyethylene glycol, polyvinyl acetate, or polyvinyl alcohol.

27. The method of claim 21, wherein X is a cytotoxic agent.

15 28. The method of claim 27, wherein X is an antimetabolic agent.

29. The method of claim 27, wherein X is a camptothecin.

30. The method of claim 27, wherein X is selected from the group
consisting of the following compounds and their derivatives: doxorubicin,
methotrexate, camptothecin, homocamptothecin, thiocolchicine, colchicine,
20 combretastatins, combretastin A-4, rhizoxin, rhizoxin-d, dolistatins, paclitaxel,
ansamitocin p3, and maytansinoids.

31. The method of claim 21, wherein Q is a somatostatin peptide.

32. The method of claim 21, wherein Q is a bombesin peptide.

33. The method of claim 21, wherein Z is Nle-D-Tyr-D-Ser.

34. The method of claim 21, wherein Z is Lys-D-Tyr-D-Ser.
35. The method of claim 21, wherein Z is Pal-D-Lys-D-Ser.
36. The method of claim 21, wherein Z is Thr-D-Tyr-D-Ser.
37. The method of claim 21, wherein the disease is selected from the
5 group consisting of: tumors of the lung, breast, brain, eye, prostate, or colon; tumors of neuroendocrine origin; and angiogenic blood vessels.

38. A peptide agent having the formula:

X-Y-Z-Q, wherein:

X is a detectable label, a chelating group, or is omitted;

- 10 Y is a peptide that increases the hydrophilic biodistribution of said agent, a hydrophilic polymer that includes a linker for X, or is omitted;

Q is a peptide having biological activity; and

Z is a linking peptide that, when bonded to Q at the N-terminus or at a compatible side-chain amino group of Q, preserves at least 50% of the said
15 biological activity of Q, Z having the formula:

C-E-F, wherein:

C is Lys, Orn, Dab, Dap, 4- NH₂-Phe, Nle, Ser, hSer, Abu, Nva, D-4-OH-Pro, or L-4-OH-Pro, or is deleted;

E and F are each independently selected from the group
20 consisting of: D-Lys, D-Tyr, D-Ser, D-4-OH-Pro, L-4-OH-Pro, 3-iodo-D-Tyr, 3-5 diiodo-D-Tyr, 3-bromo-D-Tyr, 3-5 dibromo-D-Tyr, D-Asn, D-Asp, L-Asp, D-Glu, and L-Glu; and when C and E are Lys and D-Tyr, respectively, F is not D-Tyr or D-Lys.

39. The peptide agent of claim 38, wherein said peptide agent is
25 attached to a detectable label.

40. The peptide agent of claim 39, wherein said peptide agent is indirectly attached to a detectable label.

41. The peptide agent of claim 39, wherein said peptide agent is directly attached to a detectable label.

5 42. The peptide agent of claim 39, wherein said detectable label is radioactive.

43. The peptide agent of claim 42, wherein said detectable label is an iodine, astatine, or bromine label that is attached to an amino acid of said peptide agent.

10 44. The peptide agent of claim 38, wherein X is a chelating group.

45. The peptide agent of claim 38, wherein X is omitted, and Y is lower acetylated, succinylated, maleinylated, or fumarylated.

46. The peptide agent of claim 38, wherein Y is a peptide sequence that increases the hydrophilic biodistribution of said agent.

15 47. The peptide agent of claim 46, wherein Y is of the formula:

U(V-V)_n, wherein:

U is D-Pro, L-Pro, D-4-OH-Pro, L-4-OH-Pro, Sar, or (NH₂-(CH₂)_m-COOH) where m=2-10, inclusive, or is deleted;

20 each V is independently selected from the group consisting of: D-Ser, L-Ser, D-Thr, L-Thr, D-Gln, L-Gln, D-Asn, L-Asn, D-4-OH-Pro, and L-4-OH-Pro; and

n is an integer from 1 to 50, inclusive.

48. The peptide agent of claim 47, wherein each V is independently D-Ser, L-Ser, or D-Gln.

49. The peptide agent of claim 38, wherein E is D-Tyr and F is D-Ser.
50. The peptide agent of claim 38, wherein Y is a hydrophilic polymer.
51. The peptide agent of claim 50, wherein Y is polyethyleneglycol, PHEA, or polyvinylalcohol.
52. The peptide agent of claim 38, wherein Q is a somatostatin peptide.
53. The peptide agent of claim 38, wherein Q is a bombesin peptide.
54. The peptide agent of claim 38, wherein Z is Lys-D-Tyr-D-Ser.
55. The peptide agent of claim 38, wherein Z is Lys-D-Ser-D-Ser.
56. The peptide agent of claim 38, wherein Z is Nle-D-Tyr-D-Ser.
- 10 57. A method of treating or diagnosing a disease comprising administering to a subject a therapeutically or diagnostically effective amount of a peptide agent having the formula:

X-Y-Z-Q, wherein:

X is a detectable label, a chelating group, or is omitted;

- 15 Y is a peptide that increases the hydrophilic biodistribution of said agent, a hydrophilic polymer that includes a linker for X, or is omitted;

Q is a peptide having biological activity; and

- 20 Z is a linking peptide that, when bonded to Q at the N-terminus or at a compatible side-chain amino group of Q, preserves at least 50% of the said biological activity of Q, Z having the formula:

C-E-F, wherein:

C is Lys, Orn, Dab, Dap, 4- NH₂-Phe, Nle, Ser, hSer, Abu, Nva, D-4-OH-Pro, or L-4-OH-Pro, or is deleted; and

E and F are each independently selected from the group consisting of: D-Lys, D-Tyr, D-Ser, D-4-OH-Pro, L-4-OH-Pro, 3-iodo-D-Tyr, 3-5 diiodo-D-Tyr, D-Asn, D-Asp, L-Asp, D-Glu, and L-Glu; and when C and E are Lys and D-Tyr, respectively, F is not D-Tyr or D-Lys.

5. 58. The method of claim 57, wherein said peptide agent is attached to a detectable label.

59. The method of claim 58, wherein said peptide agent is indirectly attached to a detectable label.

10. 60. The method of claim 58, wherein said peptide agent is directly attached to a detectable label.

61. The method of claim 58, wherein said detectable label is radioactive.

62. The method of claim 61, wherein said detectable label is an iodine label that is attached to an amino acid of said peptide agent.

15. 63. The method of claim 57, wherein X is a chelating group.

64. The method of claim 57, wherein X is omitted, and Y is lower acetylated, succinylated, maleinylated or fumarylated.

65. The method of claim 57, wherein Y is a peptide sequence that increases the hydrophilic biodistribution of said agent.

20. 66. The method of claim 65, wherein Y is of the formula:

U(V-V)_n, wherein:

U is D-Pro, L-Pro, D-4-OH-Pro, L-4-OH-Pro, Sar, or (NH₂-(CH₂)_m-COOH) where m=2-10, inclusive, or is deleted;

each V is independently selected from the group consisting of: D-Ser, L-Ser, D-Thr, L-Thr, D-Gln, L-Gln, D-Asn, L-Asn, D-4-OH-Pro, and L-4-OH-Pro; and

n is an integer from 1 to 50, inclusive.

5 67. The method of claim 66, wherein each V is independently D-Ser, L-Ser, or D-Gln.

68. The method of claim 57, wherein Y is a hydrophilic polymer.

69. The method of claim 68, wherein Y is polyethyleneglycol, PHEA, or polyvinylalcohol.

10 70. The method of claim 57, wherein Q is a somatostatin peptide.

71. The method of claim 57, wherein Q is a bombesin peptide.

72. The method of claim 57, wherein E is D-Tyr and F is D-Ser.

73. The method of claim 57, wherein Z is Lys-D-Tyr-D-Ser.

74. The method of claim 57, wherein Z is Lys-D-Ser-D-Ser.

15 75. The method of claim 57, wherein Z is Nle-D-Tyr-D-Ser.

76. The peptide agent of claim 2, wherein Q is bombesin and Z has the formula:

E-F, wherein:

20 E is D-Lys, D-Tyr, D-Ser, D-4-OH-Pro, L-4-OH-Pro, 3-iodo-D-Tyr, 3-5 diiodo-D-Tyr, 3-astatine-D-Tyr, 3-5 astatine-D-Tyr, 3-bromo-D-Tyr, 3-5 dibromo-D-Tyr, D-Asn, L-Asn, D-Asp, L-Asp, D-Gln, or L-Gln; and

F is D-Lys, D-Tyr, D-Ser, L-Ser, D-4-OH-Pro, L-4-OH-Pro, 3-iodo-D-Tyr, 3-5 diiodo-D-Tyr, 3-astatine-D-Tyr, 3-5 astatine-D-Tyr, 3-bromo-D-Tyr, 3-5 dibromo-D-Tyr, D-Asn, L-Asn, D-Asp, L-Asp, D-Glu, L-Glu, D-Gln, or L-Gln.

5 77. The method of claim 21, wherein Q is bombesin and Z has the formula:

E-F, wherein:

E is D-Lys, D-Tyr, D-Ser, D-4-OH-Pro, L-4-OH-Pro, 3-iodo-D-Tyr, 3-5 diiodo-D-Tyr, 3-astatine-D-Tyr, 3-5 astatine-D-Tyr, 3-bromo-D-Tyr, 10 3-5 dibromo-D-Tyr, D-Asn, L-Asn, D-Asp, L-Asp, D-Gln, or L-Gln; and

F is D-Lys, D-Tyr, D-Ser, L-Ser, D-4-OH-Pro, L-4-OH-Pro, 3-iodo-D-Tyr, 3-5 diiodo-D-Tyr, 3-astatine-D-Tyr, 3-5 astatine-D-Tyr, 3-bromo-D-Tyr, 3-5 dibromo-D-Tyr, D-Asn, L-Asn, D-Asp, L-Asp, D-Glu, L-Glu, D-Gln, or L-Gln.

15 78. The peptide agent of claim 38, wherein Q is bombesin and Z has the formula:

E-F, wherein:

E and F are each independently selected from the group consisting of: D-Lys, D-Tyr, D-Ser, D-4-OH-Pro, L-4-OH-Pro, 3-iodo-D-Tyr, 20 3-5 diiodo-D-Tyr, 3-bromo-D-Tyr, 3-5 dibromo-D-Tyr, D-Asn, D-Asp, L-Asp, D-Glu, and L-Glu.

79. The method of claim 57, wherein Q is bombesin and Z has the formula:

E-F, wherein:

E and F are each independently selected from the group
5 consisting of: D-Lys, D-Tyr, D-Ser, D-4-OH-Pro, L-4-OH-Pro, 3-iodo-D-Tyr, 3-5 diiodo-D-Tyr, 3-bromo-D-Tyr, 3-5 dibromo-D-Tyr, D-Asn, D-Asp, L-Asp, D-Glu, and L-Glu.

80. The peptide agent of claim 2, wherein Z is Lys-D-Tyr-D-Ser.

10 81. The peptide agent of claim 2, wherein Z is Ser-D-Lys-D-Ser.

82. The peptide agent of claim 2, wherein Z is Ser-D-Lys-Ser.

83. The peptide agent of claim 2, wherein Z is Nle-D-Tyr-D-Ser.

15 84. The peptide agent of claim 2, wherein Z is Pal-D-Lys-D-Ser.

85. The peptide agent of claim 2, wherein Z is Thr-D-Tyr-D-Ser.

20 86. The method of claim 21, wherein Z is D-Ser-Lys-D-Tyr-D-Tyr.

87. The method of claim 21, wherein Z is D-Ser-Lys-D-Tyr-D-Ser.

88. The method of claim 21, wherein Z is D-Ser-Ser-D-Lys-D-Ser.

25 89. The method of claim 21, wherein Z is D-Ser-Ser-D-Lys-Ser.

90. The method of claim 21, wherein Z is D-Ser-Nle-D-Tyr-D-Ser.

91. The method of claim 21, wherein Z is D-Ser-Pal-D-Tyr-D-Ser.
92. The method of claim 21, wherein Z is D-Ser-Thr-D-Tyr-D-Ser.
- 5 93. The method of claim 21, wherein Z is Ser-D-Lys-Ser.
94. The method of claim 21, wherein Z is Ser-D-Lys-D-Ser.
95. The peptide agent of claim 4, wherein at least one V is a D-amino
10 acid.
96. The method of claim 23, wherein at least one V is a D-amino acid.
97. The peptide agent of claim 47, wherein at least one V is a D-amino
15 acid.
98. The method of claim 66, wherein at least one V is a D-amino acid.
99. The method of claim 37, wherein said tumor of neuroendocrine
20 origin is carcinoid syndrome.
100. The peptide agent of claim 44, wherein said chelating group
comprises an isotope of Lu, In, Y, or Sm.
- 25 101. The method of claim 63, wherein said chelating group comprises an
isotope of Lu, In, Y, or Sm.

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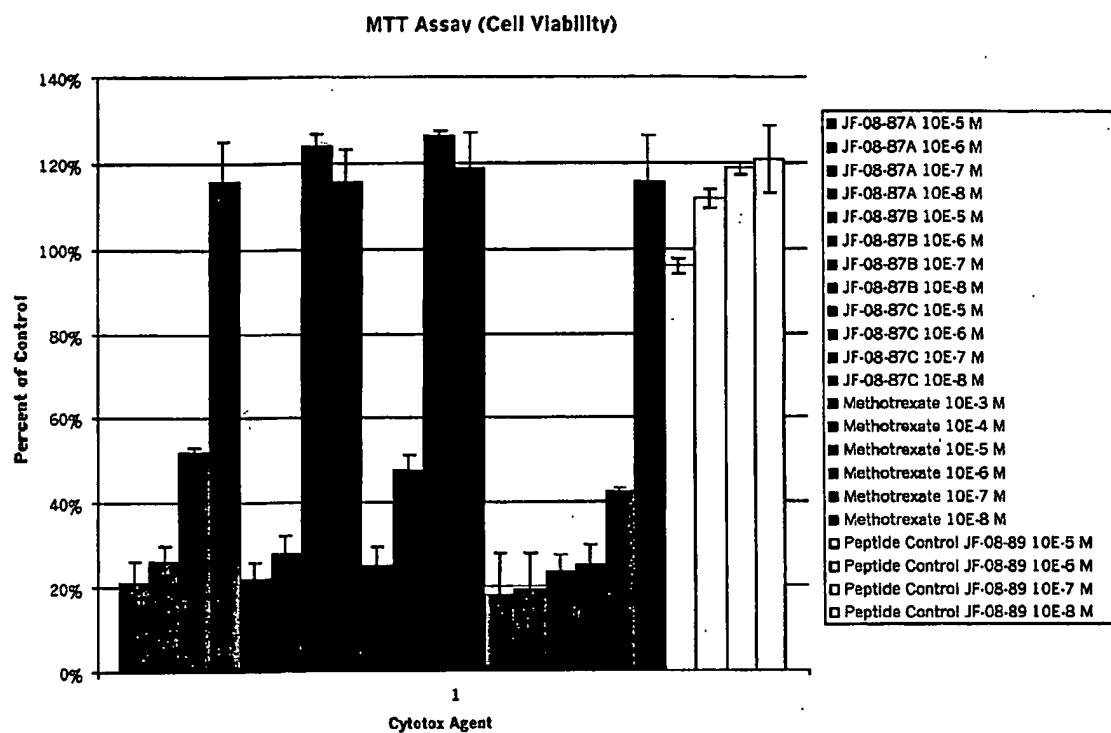


Fig. 1

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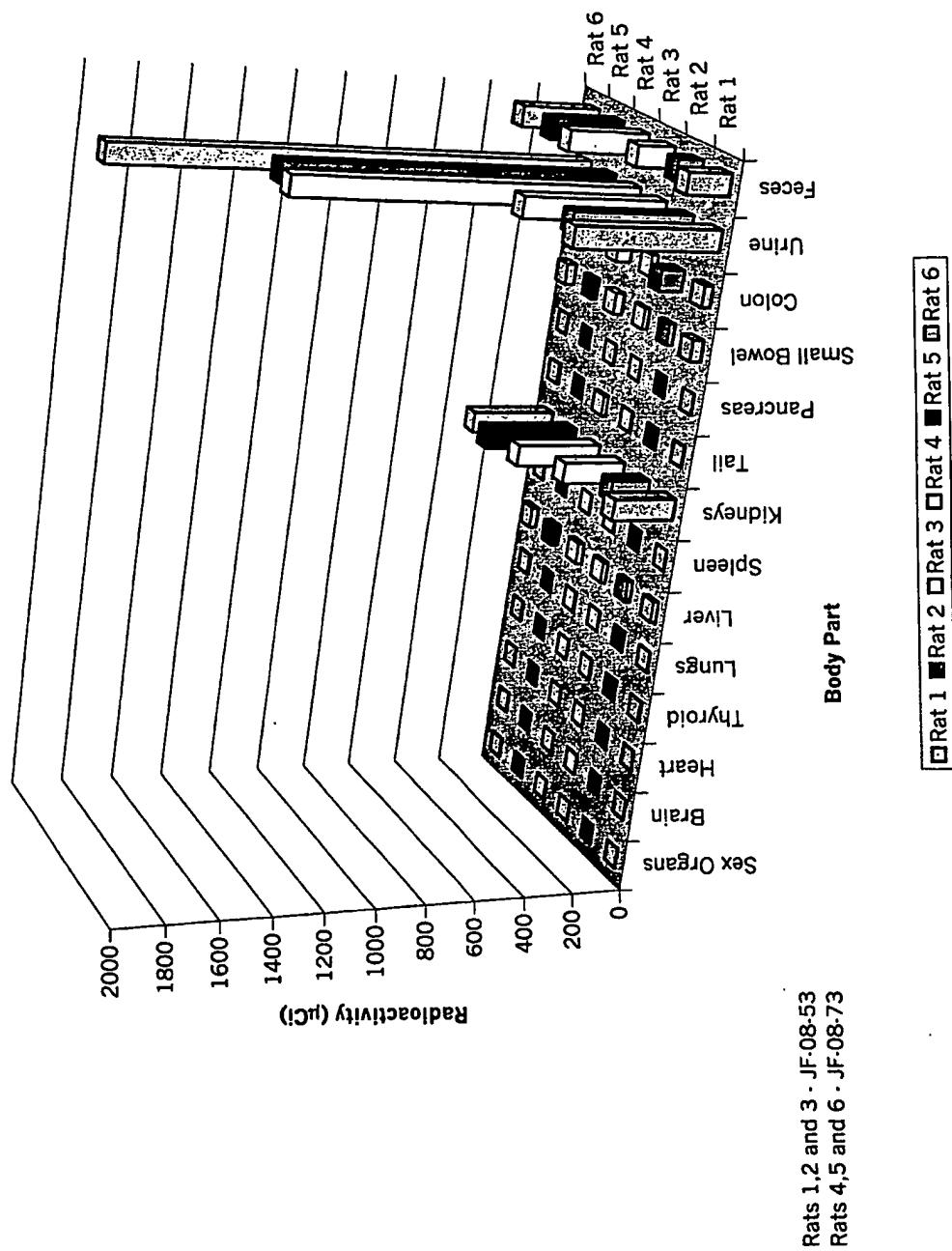
Rat Organ Distribution Study

Fig. 2

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<223> Xaa=BAla, 4Abu, Gly, Ala, D-Ala, N-Me-Ala, or N-Me-D-Ala.

<221> VARIANT
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<222> 7
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<221> VARIANT
<222> 8
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<223> Xaa at position 9 is Ava, Gly, Leu, Val, Ile, Nle, or Nva.

<221> VARIANT
<222> (10)...(10)
<223> Xaa at position 10 is Ava, Gly, Leu, Val, Ile, Nle, Nva, or a lower alkyl amide.

<223> Synthetic

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1

5

10

15

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Phe Xaa

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20

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20

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